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厌氧微生物培养分离：过去、现在和未来

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摘要：厌氧菌是地球上数量最多、物种最丰富的微生物，也是分类上报道最少的微生物。它们对氧气敏感、生长条件苛刻，不容易培养分离。本文简要总结了厌氧微生物的研究历史，分析了限制厌氧微生物培养分离的主要因素，讨论了厌氧微生物培养分离的策略和方法，回顾了国内外厌氧微生物的系统分类学现状，并展望了厌氧微生物培养分离的发展趋势。

关键词：厌氧微生物，分离培养，高通量筛选，定向分离

根据微生物对氧气的利用和耐受程度，科学家曾经将它们分为好氧、微好氧、严格厌氧、兼性厌氧和耐氧微生物(表 1)。厌氧微生物无法在有氧条件下生长。兼性厌氧微生物在有无氧条件下都可以生长繁殖。耐氧微生物则能够在厌氧条件下生长，也能耐受一定浓度的氧气。过去认为氧气浓度达到巴斯德点(Pasteur point)，即氧分压达到当前大气氧分压水平(PAL)的 1% (大约相当于 94 μmol/L)，是好氧微生物和厌氧微生物生长的分界点[1–2]。但 Baughn 等发现厌氧菌 *Bacteroides fragilis* 利用 nmol/L 级的氧气生长[3]。这类厌氧菌被定义为“纳级厌氧菌(Nanaerobe)”[3]。Stolper 等发现某些好氧微生物也可以在氧气浓度<3 nmol/L 条件下生长[4]。严格厌氧微生物对氧气的适应和生长耐受机制还有待进一步研究。

氧气在细胞内能以氧自由基(O_2/ROS)的形式存在，如 $O_2^{-\cdot}$、H_2O_2、$^{\cdot}OH$ 和 $^1O_2$，微生物细胞除了产生抗坏血酸和谷胱氨肽等小分子的抗氧化剂外，也可以产生抗氧化酶来消除氧自由基的胁迫，如超氧化物歧化酶(SOD)、过氧化氢酶(CAT)和抗氧化蛋白(PXs)[6]。McCord 等认为严格厌氧微生物不含有超氧化物歧化酶(SOD)和过氧化氢酶活性(CAT)，而耐氧微生物则能利用超氧化合物氧化酶(SOR)抵御一定浓度的氧胁迫[7]。但是 S’lesak 等分

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表 1. 根据氧气利用特征区分的微生物类型[5]

Table 1 Types of microorganism according to oxygen utilization properties[5]

Microorganism types	Oxygen required for growth	Oxygen-free for growth	Notes
Aerobe	+	–	
Microaerobe	+	–	Grow with low concentration of O_2
Strict anaerobe	–	+	O_2 is toxic to strict anaerobe
Facultative anaerobe	+	+	Grow both with O_2 or no O_2
Aerotolerant microorganism	–	+	

析了 100 种严格厌氧微生物，发现 93%的菌种中至少含有一种抗氧化酶[6]。从能量代谢的角度，厌氧微生物无法利用氧气作为末端电子受体获取 ATP，只能通过底物水平磷酸化，或者利用硫酸盐等外源电子受体进行厌氧呼吸获得能量[8]。好氧微生物通过呼吸链传递电子并获取 ATP，其中氧化酶作为关键功能酶，一度也被认为是好氧微生物独有的特征酶。Baughn 等发现厌氧菌利用细胞色素 bd 氧化酶(cytochrome *bd* oxidase)利用 nmol/L 级的氧气生长[3]。进一步研究发现很多严格厌氧菌如 *Archaeoglobus fulgidus*、*Desulfovibrio gigas*、*Methanosarcina barkeri* 和 *Moorella thermoacetica* 都含有类似的细胞色素 bd 氧化酶编码基因，可能都具有类似的低氧代谢能力[3,9–10]。因此，通过厌氧微生物基因组中是否含有抗氧化酶和氧化酶基因，也难以准确区分它们是否属于厌氧微生物[1]。

1 厌氧微生物培养分离的研究历史

早在 300 多年前，Antoni van Leeuwenhoek 在静置培养 3 周后的胡椒水中，观察到有活的微生物存在，这是最早关于厌氧微生物培养的报道[11]。19 世纪 60 年代，法国科学家 Louis Pasteur 开启了厌氧微生物学研究，首次根据微生物是否可以在有氧条件下生长，将微生物划分为好氧和厌氧微生物[12]。此外，他和合作者分离获得了第一个致病性厌氧菌，后被命名为 *Clostridium septicum*[12]。巴斯德提出的煮沸或抽真空法制备预还原厌氧培养基是经典的厌氧操作技术之一。

在 Hungate 厌氧操作技术发明以前，科学家通常通过生物法(如共培养好氧细菌的方式消耗培养基中的氧气)、物理法(如煮沸、抽真空、惰性气体吹扫)和化学法(如添加铁、硫化物和碱性焦棓酸)等制备预还原培养基[13]。在厌氧培养的时候，通常是采用深层琼脂/液体培养基进行梯度稀释培养，并结合液封(如石蜡、原油、绵羊油、凡士林)，或者橡胶塞、密封罐等方式密封[13–14]。这些操作繁琐、难以真正隔绝氧气，而且在挑取深层单菌落的过程中也容易被污染。这导致厌氧微生物研究进展缓慢，只能分离一些产芽孢厌氧菌、兼性厌氧菌或耐氧菌，如 *Clostridium*[15–16]。

1947 年，美国微生物学家 Robert E. Hungate 通过深层琼脂法分离厌氧纤维素降解菌的时候，意外发现管内壁上层的薄层固体培养基上会形成单菌落[17]。随后他提出了滚管法分离厌氧菌的操作技术[18]，经过不断的完善[19]，该方法已成为厌氧微生物分离培养的经典方法[20]。该技术核心有 3 点：利用完全密封的玻璃管培养厌氧微生物；利用高温铜柱去除惰性气体中的残余氧，在无氧惰性气体保护下制备预还原培养基、转移厌氧微生物；利用滚管法制备贴内壁的固体培养基薄层，

生长的单菌落很容易被挑取转移[18,20]。这个操作技术所需要的设备简单，并能提供严格厌氧环境。Hungate 在厌氧微生物学领域的奠基性工作，使其成为厌氧微生物学研究的先驱[21–22]。同时期有科学家开发了封闭的充满无氧气体的手套箱，实验人员可以通过手套进行厌氧操作[23–24]，这种方法虽然比 Hungate 厌氧操作技术简单，但是手套箱中的残氧含量还是高于厌氧管，早期的厌氧手套箱无法控制洁净度，容易造成交叉污染，也难以大面积推广使用。我们课题组与企业联合开发的无菌无氧控温手套箱，氧含量最低小于 10 mg/L，内部操作空间可以达到百级洁净，温度可以控制在 10–25 °C，适宜开展大规模的无菌操作[25]。

1977 年，Carl R. Woese 提出“三域学说”，即根据核糖体基因序列相似度来指示物种亲缘关系的理论[26]，完全改变了人们对微生物物种多样性的认知。在 DNA 测序技术的推动下，科学家推测全球微生物数量达到了 10^{30} 的量级，其中超过 70% 分布在陆相和海相深部缺氧沉积物中[27–28]，这表明地球上的微生物仍然以厌氧微生物为主，其物种多样性可能远远超过好氧微生物。这也表明厌氧微生物资源是尚未充分开发的一块处女地，值得微生物资源与分类学家去关注。

2 厌氧微生物培养分离新策略

2.1 限制厌氧微生物培养分离的因素

微生物分离的经典思路是“先富集后分离”，通过选择性培养基让特定功能的微生物繁殖变为优势菌，重复在固体或液体培养基上梯度稀释培养，以纯化获取单菌落。这种传统思路的局限性也非常明显：(1) 分离过程的盲目性：环境中存在大量未培养微生物，但是不知道哪些微生物会在培养过程被选择性富集。(2) 分离的随机性：不同种类的环境微生物浓度可能相差 3–5 个数量级[29–30]。即使假定所有微生物都可以培养生长，理论上也需要随机挑取数百万级别的单菌落，才有可能分离到所有的微生物。而传统 Hungate 滚管法的分离通量很难超过 2–3 个数量级，难以挑取到所有微生物。(3)菌株生长的不确定性：人工设计的培养基和培养条件无法满足所有原位微生物的生长。如磷酸盐缓冲液与琼脂高温湿热灭菌后，会抑制微生物生长[31]。有些微生物的菌体生长浓度只有 10^5 个/mL 甚至更低，难以通过选择培养的方式进行富集[32]。(4)菌株生理特征的未知性：厌氧微生物不仅生长缓慢，对氧气敏感，而且存在更为复杂的种间互作关系，如互营微生物依赖于产甲烷古菌的互营代谢关系[33]。如果不清楚这些未培养微生物的生理特征，也难以分离培养它们。

2.2 提高厌氧微生物培养分离的策略

鉴于厌氧微生物的特殊生理特征，我们总结国内外分离厌氧微生物的研究策略，主要通过测序引导分离、开发新装置提高分离效率、改善培养条件、预测未培养微生物的潜在代谢功能等策略，来提高厌氧微生物的分离效率(表 2)。

2.2.1 通过高通量测序技术等解决厌氧微生物分离过程的盲目性：通过微生物分子生态学技术，实时跟踪不同处理和培养条件下目标菌的生长状态，降低厌氧菌分离的盲目性。Sekiguchi 等采用荧光原位杂交技术(FISH)监测厌氧丝菌 UN1-1 的分离，后被鉴定为新属 *Anaerolinea*[34–35]。类似的，Sakai 设计产甲烷古菌 RC-I 的探针，用于指导产甲烷古菌新目 *Methanocella* 的分离[36]。Ma 等在

表 2. 厌氧微生物分离的策略

Table 2. Isolation strategies of anaerobic microorganisms

Isolation strategies	Isolation methods	Explanation of isolation methods	References
Development of real-time observation technology	16S rRNA and functional genes sequencing	The growth statue of targeted microorganisms was observed by 16S rRNA and functional gene sequencing	[36–37]
	FISH	Real-time monitoring the growth condition of target microorganisms through FISH	[34–35]
Development of equipment for high-throughput cultivation and identification	Microplate	Lowering cultivation volume to μL-nL and improving plates number to hundreds and thousands	[42,44]
	Microfluidics	Lowering cultivation volume to μL-nL and improving plates number to ten thousand	[37,43]
	Microencapsulation	Gradient dilution microorganisms and mixing low concentration agar to micro gel capsule and cultured in lipid medium	[45]
	Single cell sorting	Using flow cytometry or Raman spectra technique to selectively pick microorganisms	[40–41]
	MALDI-TOF	Use of protein profiles obtained by Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) to for bacteria identification	[38]
Optimization of cultivation condition	Syntrophic cultivation	Co-cultured incubation with hydrogenotrophic methanogens or syntrophic bacteria to isolate syntrophic bacteria and methanogens respectively	[36,46–47]
	Parasitic cultivation	Providing amino acids and cofactors to target microorganism by other microorganism	[48]
	Agar substitution	Using phytagel (Glen glue) to prepare solid medium	[31,49]
	Antibiotics addition	Selective inhibition of anaerobe by different antibiotics addition	[51]
	Pretreatment with ethanol	Pretreatment with ethanol to inactivate non-spore microorganisms to isolate spore-producing anaerobes	[30]
	Pre-reduced medium preparation by addition of different reductant solutions	Titanium citrate or sodium ascorbate was chosen as reductants	[52–53]
	Selective addition of electron acceptors	Selective enrichment and cultivation of different types of anaerobes through adding different electron acceptors	[29]
	Cultivation under low nutrition concentration	Reducing medium or substrate concentration by 10-100 times	[54–55]
	In-situ cultivation	Simulating in-situ environment, substance exchange and spatial separation to improve cultivability of microorganisms	[70]
	Continuous flow cultivation	Isolation and cultivation of high abundance uncultured microorganism through continuous dynamic cultivation	[56–57]
Culturomics	High throughput isolation using different medium and incubation conditions	High throughput isolation using different medium and incubation conditions	[39,66]
“Cocktail method”	Treatment with multiple isolation methods	Enrichment of targeted anaerobes by continuous flow cultivation, and cocultured incubation under low H_2 pressure and low nutrition conditions	[67]
Directional isolation	Reverse genome method	Using labelled antibody according to the speculating epitope from the membrane protein gene of uncultured microorganisms to screen and cultivate specific binding microorganism	[69]

16S rRNA 基因序列的引导下，结合滑动微流控芯片技术分离获得 *Ruminococcaceae* 的一个新属[37]。利用 MALDI-TOF 技术分析菌株核糖体蛋白的差异特征[38–39]，菌株鉴定通量可以达到几百个菌株/小时，并能鉴定到种或属水平，这样能降低后续50%–80%的工作量。Witkowska 等通过表面增强拉曼光谱技术可以识别菌株水平的微生物[40]。Bellais 等通过特异性多克隆抗体反应，通过流式细胞仪识别和分选出来肠道 *Faecalibacterium prausnitzii*[41]。

2.2.2 开发新装置和设备提高厌氧微生物分离的通量：随着高通量培养装备和检测技术的发展，将单个或少数几个微生物细胞分散到单孔中，也可以进行“先分离后富集”。开发厌氧微生物高通量分离培养装置，降低“培养瓶”的容积，可以提高微生物的分离效率。有科学家利用 96 或 384 微孔板，将培养体积降低到 100–200 μL，相应的，“培养瓶”的数量能提高到数千个量级[32,42]。微流控技术的应用进一步将培养孔的体积降低到 nL 甚至 pL 尺度。Ma 等设计的微流控滑动芯片有 3200 个微孔能用于微生物培养，每孔容积只有 6 nL 左右[37]。Villa 等应用微流控液滴技术，在厌氧手套箱中分离肠道厌氧微生物[43]。Ingham 等构建的半透性芯片，最高可以布置近百万个芯片微孔，一次性可以扫描 20 多万个单菌[44]。Zengler 等利用低浓度琼脂包裹的凝胶微囊，可以在流动性培养液中培养单菌[45]。

2.2.3 通过优化培养条件改善厌氧微生物的可培养性：添加伴生菌可以促进厌氧微生物的生长。如互营微生物是一类独特厌氧微生物，需要伴生菌消耗氢气并维持低氢分压才能生长。Qiu 等通过添加外源氢营养型产甲烷古菌，分离获得了互营苯酚降解菌新属 *Syntrophorhabdus*[46]。同时，他们课题组反其道而行之，通过互营丙酸降解菌产生的低浓度氢气，来“钓取”水稻根际甲烷排放的重要未培养产甲烷古菌新目 *Methanocellales*[36–47]。Huber 等发现嗜热古菌新门 *Cadidaus Nanoarchaeota* 依赖于嗜热化能自养古菌 *Ignicoccus* 才能生长[48]。Carbonero 等发现难以在普通琼脂培养上生长的产甲烷古菌 *Methanothrix* (原属名 *Methanosaeta*)，可以在 0.3%结冷胶培养基上形成菌落[49]。国外科学家发现磷酸盐缓冲液与琼脂分开灭菌后可以提高微生物的可培养性[31,49]，我们研究也发现磷酸盐缓冲液会增加乙酸代谢产甲烷的延滞期，降低古菌群落的多样性[50]。添加抗生素选择性抑制厌氧微生物[51]，添加乙醇获得产芽孢的厌氧菌[30]，添加硫酸盐、铁锰氧化物作为电子受体，或添加不同的还原剂，如柠檬酸钛和抗坏血酸钠，也可以选择性富集不同类型的厌氧还原菌[29,52–53]。这些都可以提高厌氧微生物新物种的分离概率[52–53]。此外，通过稀释培养基、降低培养基成分的浓度[54–55]，在连续流反应器中动态驯化培养[56–57]，也分离获得了厌氧微生物新种属[57]。

2.2.4 通过宏组学技术预测未培养微生物的代谢功能：高通量测序和生物信息学技术的快速发展，可以跳过微生物培养环节，拼接获得未培养微生物的全基因组序列，根据基因组序列特征推测它们的遗传代谢潜力。如 Evans 等直接提取地下煤层水中的总 DNA，通过二代测序技术获得了宏基因组序列，再根据 GC 含量、片段测序深度和四核苷酸多态性等序列特征，组装拼接出 2 个未培养古菌的基因组序列。进一步分析发现这 2 个基因组中都含有依赖 H_2 的甲基裂解产甲烷途径的编码基因，它们属于 *Bathyarchaeota*(深古菌门)，而

不是传统认为的 *Euryarchaeota*[58]。类似的，在 *Verstraetearchaeota*、*Nezhaarchaeota*、*Korarchaeota* 和 *Thaumarchaeota* 等未培养新门中都发现了新型产甲烷古菌[59–62]。通过富集培养、宏转录组测序和中间代谢产物分析，能发现未培养微生物的代谢新功能，如完全硝化细菌、降解短链烷烃的硫酸盐还原细菌和古菌等[54,63–65]。标记培养和显微成像技术，如 CARD-FISH 和 NanoSIMS 结合，可以在单细胞水平鉴别未培养古菌的碳代谢功能。Chen 等发现古菌 *Ca. Argoarchaeum* 细胞内的硫含量显著高于其他硫酸盐还原菌，推测原位硫酸盐还原降解乙烷主要发生在古菌而不是细菌细胞中[65]。

由于微生物物种的多样性和生理特征的异质性，难以通过单一的策略和方法分离所有的微生物。组合多种培养基和培养条件，进行高通量分离筛选的“培养组学”应运而生[39,66]。如 Lagier 等尝试了 212 种培养条件，从 12 个粪便样品中分离挑取了 90 万个单菌落，结合 MALDL-TOF 和 16S rRNA 基因测序，共获得了 1057 种微生物(其中新种以上的分类单元近 200 个)[39]。另外，Imachi 等通过连续流驯化、共培养降低氢分压、低浓度生长和特异性序列监测的“鸡尾酒”法(综合多种分离策略和方法)，并努力和坚持数十年，分离出厌氧古菌新门 *Asgard* 的纯培养物[67]。该研究可能会改写生命起源与进化认知[68]。Cross 等通过宏基因组或单细胞测序技术获得未培养微生物的基因组序列，并预测未培养微生物的膜蛋白基因预测抗原表位，通过制备特异性的标记抗体，来特异性结合并筛选目标细菌，从而进行后续培养[69]。如果这个技术具有普适性，那么将突破活体微生物细胞的定向预分离技术，为后续的分离和原位生理功能研究奠定基础。

3 已分离厌氧微生物的物种多样性

截止本文修订时，全球有效发表的原核微生物(具有正确的拉丁文名)只有 17304 个种，分别属于 38 门、73 纲、213 目、52 科、3563 属(截止 2020-8-15，https://lpsn.dsmz.de/text/numbers)，其中厌氧微生物只有 2151 个种，低于好氧微生物一个数量级。科学家推测原核微生物的种水平多样性可能高达 10^6–10^{12} 个[71–74]。因此，从全球尺度看，可培养微生物比例不超过 1%，其中厌氧纯培养物的可培养度不及 0.1%，绝大部分厌氧微生物处于未培养状态。

20 世纪 80 年代，我国开始厌氧微生物的分离培养，特别是产甲烷古菌的分离培养研究[75–80]。直到 2003 年，我国科学家(第一作者或通讯作者单位为国内科研机构)才首次正式提出厌氧微生物新物种[81]。截止 2020 年 8 月，国内科学家共提出 92 个新种，分布于 11 门、18 纲、29 目、47 科、71 属(表 3)。其中，穆大帅等发表的新纲 *Tichowtungiia* 是目前我国科学家提出的厌氧微生物的最高分类单元[82]。另外，我国科学家还提出了 2 个新目(*Thermosediminibacterales* 和 *Moorellales*)、9 个新科，除中山大学李文均课题组提出的新科 *Xylanivirgaceae* 外[83]，其他均由我们课题组提出[84–86]。在 92 个新种中，只有 9 个属于古菌域，其中 7 个属于广古菌门，主要由东秀珠研究员和我们课题组提出，东老师课题组发现乙酸营养型产甲烷古菌新种 *Methanothrix harundinacea*[87]，后被证实具有利用电子还原二氧化碳产甲烷的功能[88]。我们课题组发现的甲基营养型产甲烷古菌新科 *Methermicoccaceae*[85]，后被证实具有直接降解煤炭中氧甲基化合物产甲烷的功能[89]。

表 3. 国内报道的厌氧微生物新物种

Table 3. Novel taxa of anaerobic microorganisms proposed by Chinese scholars

Domain	Phylum	Class	Order	Family	Genus	Species	References
Archaea	*Euryarchaeota*	*Methanobacteria*	*Methanobacteriales*	*Methanobacteriaceae*	*Methanobacterium*	*Methanobacterium beijingense*	[90]
					Methanothermobacter	*Methanothermobacter crinale*	[91]
			Methanomicrobiales	*Methanomicrobiaceae*	*Methanoculleus*	*Methanoculleus receptaculi*	[92]
						Methanoculleus hydrogenitrophicus	[93]
			Methanosarcinales	*Methanosaetaceae*	*Methanosaeta*	*Methanosaeta harundinacea*	[87]
				Methermicoccaceae	*Methermicoccus*	*Methermicoccus shengliensis*	[85]
				Methanosarcinaceae	*Methanolobus*	*Methanolobus psychrophilus*	[94]
		Thermococci	*Thermococcales*	*Thermococcaceae*	*Palaeococcus*	*Palaeococcus pacificus*	[95]
					Thermococcus	*Thermococcus eurythermalis*	[96]
Bacteria	*Actinobacteria*	*Actinobacteria*_c	*Actinomycetales*	*Actinomycetaceae*	*Actinomyces*	*Actinomyces ruminicola*	[97]
			Micrococcales	*Micrococcaceae*	*Glutamicibacter*	*Glutamicibacter ardleyensis*	[98–99]
				Microbacteriaceae	*Microbacterium*	*Microbacterium nanhaiense*	[100]
	Aquificae	*Aquificae*_c	*Aquificales*	*Desulfurobacteriaceae*	*Desulfurobacterium*	*Desulfurobacterium indicum*	[101]
	Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Acetobacteroides*	*Acetobacteroides*	*Acetobacteroides hydrogenigenes*	[102]
				Porphyromonadaceae	*Paludibacter*	*Paludibacter jiangxiensis*	[103]
					Parabacteroides	*Parabacteroides chartae*	[104]
						Parabacteroides acidifaciens	[105]
					Proteiniphilum	*Proteiniphilum acetatigenes*	[106]
			Marinilabiliales	*Marinifilaceae*	*Ancylomarina*	*Ancylomarina psychrotolerans*	[107]
						Ancylomarina longa	[108]
				Marinilabiliaceae	*Mangroviflexus*	*Mangroviflexus xiamenensis*	[109]
					Thermophagus	*Thermophagus xiamenensis*	[110]
	Firmicutes	*Clostridia*	*Clostridiales*	*Anaerovirgula*	*Anaeromicrobium*	*Anaeromicrobium sediminis*	[111]
					Inediibacterium	*Anaerophilus nitritogenes*	[112]
					Salimesophilobacter	*Salimesophilobacter vulgaris*	[113]
					Wukongibacter	*Wukongibacter baidiensis*	[114]

(待续)

续表 3

	Clostridiaceae	*Clostridium*	*Clostridium algifaecis*	[115]
			Clostridium amylolyticum	[116]
			Clostridium beihaiense	[117]
			Clostridium bovifaecis	[118]
			Clostridium fermenticellae	[119]
			Clostridium guangxiense	[120]
			Clostridium huakuii	[121]
			Clostridium liquoris	[122]
			Clostridium luticellarii	[123]
			Clostridium neuense	[120]
			Clostridium prolinivorans	[124]
			Clostridium swellfunianum	[125]
		Haloimpatiens	*Haloimpatiens lingqiaonensis*	[126]
		Oceanirhabdus	*Oceanirhabdus sediminicola*	[127]
		Proteiniclasticum	*Proteiniclasticum ruminis*	[128]
	Defluviitaleaceae	*Defluviitalea*	*Defluviitalea phaphyphila*	[129]
		Defluviitalea	*Defluviitalea raffinosedens*	[130]
	Lachnospiraceae	*Cellulosilyticum*	*Cellulosilyticum ruminicola*	[131]
	Natronincola	*Alkaliphilus*	*Alkaliphilus crotonatoxidans*	[81]
			Alkaliphilus halophilus	[132]
	Oscillospiraceae	*Acetanaerobacterium*	*Acetanaerobacterium elongatum*	[133]
		Ethanoligenens	*Ethanoligenens harbinense*	[134]
		Hydrogenoanaerobacterium	*Hydrogenoanaerobacterium saccharovorans*	[135]
		Petroclostridium	*Petroclostridium xylanilyticum*	[84]
		Saccharofermentans	*Saccharofermentans acetigenes*	[136]
	Peptococcaceae	*Desulfotomaculum*	*Desulfotomaculum ferrireducens*	[137]
	Peptostreptococcaceae	*Romboutsia*	*Romboutsia sedimentorum*	[138]
		Sporacetigenium	*Sporacetigenium mesophilum*	[139]
		Terrisporobacter	*Terrisporobacter petrolearius*	[140]
	Syntrophomonadaceae	*Syntrophomonas*	*Syntrophomonas cellicola*	[141]
			Syntrophomonas curvata	[142]
			Syntrophomonas erecta	[143]
		Thermosyntropha	*Thermosyntropha tengcongensis*	[144]

(待续)

续表 3

			Thermohalobacter	*Brassicibacter*	*Brassicibacter mesophilus*	[145]
					Brassicibacter thermophilus	[146]
				Caloranaerobacter	*Caloranaerobacter ferrireducens*	[147]
				Proteiniborus	*Proteiniborus ethanoligenes*	[148]
			Vallitaleaceae	*Vallitalea*	*Vallitalea okinawensis*	[149]
			Xylanivirgaceae	*Xylanivirga*	*Xylanivirga thermophila*	[83]
		Eubacteriales	*Eubacteriaceae*	*Peptacetobacter*	*Peptacetobacter hominis*	[150]
				Rhabdanaerobium	*Rhabdanaerobium thermarum*	[151]
		Halanaerobiales	*Anoxybacter*	*Anoxybacter*	*Anoxybacter fermentans*	[152]
		Moorellales	*Zhaonellaceae*	*Zhaonella*	*Zhaonella formicivorans*	[86]
		Thermoanaerobacterales	*Thermoanaerobacterium*	*Thermoanaerobacterium*	*Thermoanaerobacterium calidifontis*	[153]
		Thermosediminibacterales	*Tepidanaerobacteraceae*	*Biomaibacter*	*Biomaibacter acetigenes*	[154]
	Negativicutes	*Hydrogenispora_o*	*Hydrogenispora*	*Hydrogenispora*	*Hydrogenispora ethanolica*	[155]
		Selenomonadales	*Selenomonadaceae*	*Mitsuokella*	*Selenomonas bovis*	[156]
				Pectinatus	*Pectinatus brassicae*	[157]
	Tissierellia	*Tissierellales*	*Tissierellaceae*	*Gudongella*	*Gudongella oleilytica*	[158]
				Keratinibaculum	*Keratinibaculum paraultunense*	[159]
				Tepidimicrobium	*Tepidimicrobium xylanilyticum*	[160]
			Sedimentibacter	*Sedimentibacter*	*Sedimentibacter hongkongensis*	[161]
Kiritimatiellaeota	*Tichowtungiia*	*Tichowtungiales*	*Tichowtungiaceae*	*Tichowtungia*	*Tichowtungia aerotolerans*	[82]
Planctomycetes	*Planctomycetia*	*Planctomycetales*	/	*Thermopirellula*	*Thermopirellula anaerolimosa*	[162]
Proteobacteria	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*	*Geobacter anodireducens*	[163]
					Geobacter soli	[164]
		Desulfovibrionales	*Desulfovibrionaceae*	*Pseudodesulfovibrio*	*Pseudodesulfovibrio indicus*	[165]
		Syntrophobacterales	*Syntrophobacteraceae*	*Syntrophobacter*	*Syntrophobacter sulfatireducens*	[166]
	Epsilonproteobacteria	*Campylobacterales*	*Sulfurimonas*	*Sulfurimonas*	*Sulfurimonas hongkongensis*	[167]
	Gammaproteobacteria	*Alteromonadales*	*Shewanellaceae*	*Shewanella*	*Shewanella decolorationis*	[168]
Synergistetes	*Synergistia*	*Synergistales*	*Synergistaceae*	*Lactivibrio*	*Lactivibrio alcoholicus*	[169]
Thermodesulfobacteria	*Thermodesulfobacteria_c*	*Thermodesulfobacteriales*	*Thermodesulfobacteriaceae*	*Thermodesulfatator*	*Thermodesulfatator autotrophicus*	[170]
Thermotogae	*Thermotogae_c*	*Kosmotogales*	*Kosmotogaceae*	*Kosmotoga*	*Kosmotoga shengliensis*	[172–173]
					Kosmotoga pacifica	[173]
		Thermotogales	*Fervidobacteriaceae*	*Fervidobacterium*	*Fervidobacterium changbaicum*	[174]
Verrucomicrobia	*Spartobacteria*	*Chthoniobacterales*	*Chthoniobacteraceae*	*Terrimicrobium*	*Terrimicrobium sacchariphilum*	[175]

厌氧微生物生长条件苛刻，对保藏技术和设施要求较高。目前，厌氧微生物模式物种主要保藏在德国、美国和日本(表 4)。其中，德国 DSMZ 收集保藏的厌氧微生物模式物种达到了 1896 个，是国际上最大的厌氧微生物模式物种保藏机构。2003 年在“国家科技基础条件平台建设项目”的资助下，我国厌氧微生物资源保藏工作开始起步。目前，国内已保藏厌氧微生物 1663 株，模式物种 515 个(表 5，不同保藏中心可能存在相同物种)。依托农业部沼气科学研究所的中国厌氧微生物资源管理中心(前身为农业部厌氧微生物重点开放实验室)，是国际菌种联盟(WFCC)的会员单位，具备新物种的保藏资质，目前已保藏厌氧微生物模式菌株 334 种，产甲烷古菌 45 种，厌氧微生物模式物种保藏量国内领先，在国际上排名第四(表 4 和 5)。

4 展望

在上世纪 60 年代，知名微生物学家 Roger Stanier 悲观地认为“对细菌分类是无法实现的科学目标”[176]。但是 Carl R. Woese 根据核酸序列相似性提出的“三域学说”理论，近乎完美地解决了微生物的系统分类学问题[26]。基于这个思路揭示了地球上丰富的微生物多样性，发现了海量的“微生物暗物质”[28,178]。这奠定了现代环境微生物学研究的基础，并开创了微生物分子生态学研究。当然，新认知也产生新的科学问题——微生物数量高达 10^{30}，物种数可能超过百万级的“微生物暗物质”是否可以被分离培养？笔者长期从事厌氧微生物资源与系统分类学研究，过去我们主要基于生理和形态学特征，并结合多种手段开展厌氧微生物的分离。例如，我们富集获得了 65 °C 条件下降解甲醇产甲烷的富集培养物，但是没有如此高温甲基营养型产甲烷古菌的报道。因此，我们选择采用 Hungate 固体滚管技术，并降低到 55 °C 培养 1–2 个月后挑取单菌落。从而顺利分离并鉴定到 *Methermicoccus shengliensis* ZC-1[85]。后来采用先“分离”后“培养”的思路，先将菌液分分散到微孔中，再尝试添加不同类型的培养基进行

表 4. 全球厌氧微生物模式物种收集保存现状

Table 4. Summary of Anaerobic type strains in major culture collecting centers

Affiliations	Abbreviations	Type strain (Species)
German Collection of Microorganisms and Cell Cultures	DSMZ	1896
Japan Collection of Microorganisms	JCM	866
American Type Culture Collection	ATCC	844
China Collection of Anaerobic Microorganisms[a]	CCAM	334
Collection de L'Institut Pasteur Of Institut Pasteur	CIP	228
Belgian Co-ordinated Collections of Microorganisms	LMG/BCCM	137
Korean Collection for Type Cultures	KCTC	126
NITE Biological Resource Center	NBRC	122
Bioresources Collection and Research Center	BCRC	52
China General Microbiological Culture Collection Center[c]	CGMCC	51
Culture Collection University of Gothenburg[b]	CCUG	43

[a] Data from Global Catalogue Microorganisms website: http://gcm.wfcc.info/cc/ccam. [b] Data from CCUG website: https://www.ccug.se/collections/search?t=ANAEROBIC&collection=typestrains. [c] 47 type strains were collected from https://lpsn.dsmz.de/, another 4 (*Methanolobus vulcani*, *M. oregonensis*, *M. bombayensis* and *Methanobacterium flexile*) were from http://www.cgmcc.net/directory/index.html. Other data from LPSN website: https://lpsn.dsmz.de/

表 5. 我国厌氧微生物模式物种保藏现状

Table 5. Type species conservation of anaerobic microorganisms in mainland of China

Organization names	Supporting institution	Anaerobes		Methanogens		Main anaerobes resources	Data sources
		Strain number	Type strain (species)	Strain number	Type strain (species)		
China Collection of Anaerobic Microorganisms (CCAM)	Biogas Institute of Ministry of Agriculture	630	334	73	45	Fermentative bacteria, Sulfate reducing bacteria, syntrophic bacteria and methanogens etc.	http://gcm.wfcc.info/cc/ccam
China Center of Industrial Culture Collection (CICC)	China National Research Institute of Food and Fermentation	309	19	0	0	Clostridia, *Bifidobacterium*	http://sales.china-cicc.org/category.php?id=1&sh=go
Agricultural Culture Collection of China (ACCC)[#]	China Institute of Agricultural Resources and Regional Planning	299	17	111	10	Methanogens and Anaerobic fermentation system and oil deposit	Data from ACCC
China General Microbiological Culture Collection Center (CGMCC)	Microbiology Research Institute of China Science Academy	143	51	12	11	Methanogens, fermentative bacteria	http://www.nimr.org.cn/page/search/search1.jsphttps://lpsn.dsmz.de/ http://www.cgmcc.net/directory/mulu.php?number=&genus=&species=&yiming=%E7%94%B2%E7%83%B7&p=1
Guangdong Microbial Culture Collection Center (GDMCC)	Guangdong Institute of Microbiology	168	48	0	0	Anaerobes form animal oral, gut or faeces	Data from GDMCC
National Center For Medical Culture Collections (CMCC)	National Institutes for Food and Drug Control	77	14	0	0	*Clostridium tetani*	http://www.nimr.org.cn/page/search/search1.jsp
China Center for Type Culture Collection (CCTCC)	Wuhan university	18	12	0	0	*Bifidobacterium Saccharomyces*	http://www.nimr.org.cn/page/search/search1.jsp
Marine Culture Collection of China (MCCC)	Third Institute of Oceanography, Ministry of Natural Resources	14	14	0	0	Marine anaerobes: hot aureus、oil spirochete etc.	Data from MCCC
China Pharmaceutical Culture Collection (CPCC)	Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College	5	5	0	0	Anaerobes from animal faeces	Data from CPCC

The total only included anaerobic microorganisms, but not included aerotolerant and facultatively anaerobic microorganisms. #: Most of anaerobes come from CCAM.

富集培养，也先后获得了多个新属[86,155]。但是，如果采用这些传统思路和方法，根据全球新物种的分离鉴定速度(10^{2-3} 量级/年)来推测，那么似乎这又是“无法实现的科学目标”。

然而笔者相信，利用多学科交叉融合研发出的智能装备，开展厌氧微生物的全自动培养和分离将是未来的发展趋势；从群落、细胞、基因等多维度收集获得的大数据，用于训练开发人工智能，设计适用于未培养微生物的培养基和生长条件，动态监控并调整未培养微生物的生长状态，快速鉴定未培养微生物的功能。在微尺度、近原位和单细胞水平的微生物功能研究，将把传统的实验浓缩到一个芯片上完成。面对生长缓慢、对氧气敏感、营养条件苛刻的厌氧微生物的分离培养，耐心和坚持也是至关重要的。

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参 考 文 献

[1] Ślesak I, Kula M, Ślesak H, Miszalski Z, Strzałka K. How to define obligatory anaerobiosis? An evolutionary view on the antioxidant response system and the early stages of the evolution of life on Earth. *Free Radical Biology and Medicine*, 2019, 140: 61–73.

[2] Rutten MG. The history of atmospheric oxygen. *Space Life Sciences*, 1970, 2(1): 5–17.

[3] Baughn AD, Malamy MH. The strict anaerobe *Bacteroides fragilis* grows in and benefits from nanomolar concentrations of oxygen. *Nature*, 2004, 427(6973): 441–444.

[4] Stolper DA, Revsbech NP, Canfield DE. Aerobic growth at nanomolar oxygen concentrations. *Proceedings of the National Academy of Sciences of the United States of America*, 2010, 107(44): 18755–18760.

[5] Madigan MTMJ, Bender KS, Buckley DH, Stahl DA. Brock biology of microorganisms. USA: Pearson Education, 2010.

[6] Ślesak I, Ślesak H, Zimak-Piekarczyk P, Rozpądek P. Enzymatic antioxidant systems in early anaerobes: theoretical considerations. *Astrobiology*, 2016, 16(5): 348–358.

[7] McCord JM, Keele BB, Fridovich I. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. *Proceedings of the National Academy of Sciences of the United States of America*, 1971, 68(5): 1024–1027.

[8] Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. *Microbiology and Molecular Biology Reviews,* 1977, 41(1): 100–180.

[9] Lemos RS, Gomes CM, Santana M, LeGall J, Xavier AV, Teixeira M. The 'strict' anaerobe *Desulfovibrio gigas* contains a membrane-bound oxygen-reducing respiratory chain. *FEBS Letters*, 2001, 496(1): 40–43.

[10] Das A, Silaghi-Dumitrescu R, Ljungdahl LG, Kurtz DM Jr. Cytochrome *bd* oxidase, oxidative stress, and dioxygen tolerance of the strictly anaerobic bacterium *Moorella thermoacetica*. *Journal of Bacteriology*, 2005, 187(6): 2020–2029.

[11] Gest H. The discovery of microorganisms by Robert Hooke and Antoni Van Leeuwenhoek, fellows of the royal society. *Notes and Records of the Royal Society of London*, 2004, 58(2): 187–201.

[12] Sebald M, Finegold SM. Pasteur: the anaerobic life and the anaerobes. *Clinical Infectious Diseases*, 1995, 20(S2): S111.

[13] Hall IC. A review of the development and application of physical and chemical principles in the cultivation of obligately anaerobic bacteria. *Journal of Bacteriology*, 1929, 17(4): 255–301.

[14] Miller NJ, Garrett OW, Prickett PS. Anaerobic technique–a modified deep agar shake. *Journal of Food Science*, 1939, 4(5): 447–451.

[15] Cowles PB, Rettger LF. Isolation and study of an apparently widespread cellulose-fermenting anaerobe, *Cl. cellulosolvens* (N. Sp.?). *Journal of Bacteriology*, 1931, 21(3): 167–182.

[16] Hungate RE. Studies on cellulose fermentation: I. The culture and physiology of an anaerobic cellulose-digesting bacterium. *Journal of Bacteriology*, 1944, 48(5): 499-513.

[17] Hungate RE. Studies on cellulose fermentation: III. The culture and isolation for cellulose-decomposing bacteria from the rumen of cattle. *Journal of Bacteriology*, 1947, 53(5): 631–645.

[18] Hungate RE. The anaerobic mesophilic cellulolytic bacteria. *Biological Reviews*, 1950, 14(1): 1–49.

[19] Hungate RE, Smith W, Clarke RTJ. Suitability of butyl rubber stoppers for closing anaerobic roll culture tubes. *Journal of Bacteriology*, 1966, 91(2): 908–909.

[20] Hungate RE. A roll tube method for cultivation of strict anaerobes. *Methods in Microbiology*, 1969, 3: 117–132.

[21] Bryant MP. Commentary on the Hungate technique for culture of anaerobic bacteria. *The American Journal of Clinical Nutrition*, 1972, 25(12): 1324–1328.

[22] Chung KT, Bryant MP. Robert E. hungate: pioneer of anaerobic microbial ecology. *Anaerobe*, 1997, 3(4): 213–217.

[23] Drasar BS. Cultivation of anaerobic intestinal bacteria. *The Journal of Pathology and Bacteriology*, 1967, 94(2): 417–427.

[24] Aranki A, Syed SA, Kenney EB, Freter R. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. *Applied and Environmental Microbiology*, 1969, 17(4): 568–576.

[25] 承磊, 周正, 高翔. 一种无菌厌氧型生物安全柜. 中国: CN201620827463.X. 2017-01-11.

[26] Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, 1977, 74(11): 5088–5090.

[27] Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, 1998, 95(12): 6578–6583.

[28] Flemming HC, Wuertz S. Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology*, 2019, 17(4): 247–260.

[29] Köpke B, Wilms R, Engelen B, Cypionka H, Sass H. Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Applied and Environmental Microbiology*, 2005, 71(12): 7819–7830.

[30] Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, Goulding D, Lawley TD. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature*, 2016, 533(7604): 543–546.

[31] Tamaki H, Hanada S, Sekiguchi Y, Tanaka Y, Kamagata Y. Effect of gelling agent on colony formation in solid cultivation of microbial community in lake sediment. *Environmental Microbiology*, 2009, 11(7): 1827–1834.

[32] Yang SJ, Kang I, Cho JC. Expansion of cultured bacterial diversity by large-scale dilution-to-extinction culturing from a single seawater sample. *Microbial Ecology*, 2016, 71(1): 29–43.

[33] Zhang X, Zhang H, Cheng L. Key players involved in methanogenic degradation of organic compounds: progress on the cultivation of syntrophic bacteria. *Acta Microbiologica Sinica*, 2019, 59(2): 211–223. (in Chinese)
张雪, 张辉, 承磊. 获取有机物厌氧降解产甲烷过程中关键功能类群——互营细菌培养物. 微生物学报, 2019, 59(2): 211–223.

[34] Sekiguchi Y, Takahashi H, Kamagata Y, Ohashi A, Harada H. In situ detection, isolation, and physiological properties of a thin filamentous microorganism abundant in methanogenic granular sludges: a novel isolate affiliated with a clone cluster, the green non-sulfur bacteria, subdivision I. *Applied and Environmental Microbiology*, 2001, 67(12): 5740–5749.

[35] Sekiguchi Y, Yamada T, Hanada S, Ohashi A, Harada H, Kamagata Y. *Anaerolinea thermophila* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain Bacteria at the subphylum level. *International Journal of Systematic and Evolutionary Microbiology*, 2003, 53(6): 1843–1851.

[36] Sakai S, Imachi H, Sekiguchi Y, Ohashi A, Harada H, Kamagata Y. Isolation of key methanogens for global methane emission from rice paddy fields: a novel isolate

affiliated with the clone cluster rice cluster I. *Applied and Environmental Microbiology*, 2007, 73(13): 4326–4331.

[37] Ma L, Kim J, Hatzenpichler R, Karymov MA, Hubert N, Hanan IM, Chang EB, Ismagilov RF. Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa. *Proceedings of the National Academy of Sciences of the United States of America*, 2014, 111(27): 9768–9773.

[38] Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ, Lay JO Jr. Rapid identification of intact whole bacteria based on spectral patterns using matrix‐assisted laser desorption/ionization with time‐of‐flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 1996, 10(10): 1227–1232.

[39] Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, Caputo A, Cadoret F, Traore SI, Seck EH, Dubourg G, Durand G, Mourembou G, Guilhot E, Togo A, Bellali S, Bachar D, Cassir N, Bittar F, Delerce J, Mailhe M, Ricaboni D, Bilen M, Nieko NPMD, Dia Badiane NM, Valles C, Mouelhi D, Diop K, Million M, Musso D, Abrahão J, Azhar EI, Bibi F, Yasir M, Diallo A, Sokhna C, Djossou F, Vitton V, Robert C, Rolain JM, La Scola B, Fournier PE, Levasseur A, Raoult D. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nature Microbiology*, 2016, 1(12): 16203.

[40] Witkowska E, Korsak D, Kowalska A, Janeczek A, Kamińska A. Strain-level typing and identification of bacteria–a novel approach for SERS active plasmonic nanostructures. *Analytical and Bioanalytical Chemistry*, 2018, 410(20): 5019–5031.

[41] Bellais S, Nehlich M, Duquenoy A, Ania M, Van Den Engh G, Baijer J, Belotserkovsky I, Thomas V. Sorting and cultivation of *Faecalibacterium prausnitzii* from fecal samples using flow cytometry in anaerobic conditions. *bioRxiv*, 2020, doi: 10.1101/2020.03.25.007047.

[42] Colin Y, Goñi-Urriza M, Caumette P, Guyoneaud R. Contribution of enrichments and resampling for sulfate reducing bacteria diversity assessment by high-throughput cultivation. *Journal of Microbiological Methods*, 2015, 110: 92–97.

[43] Villa MM, Bloom RJ, Silverman JD, Durand HK, Jiang SR, Wu AC, Huang SQ, You LC, David LA. High-throughput isolation and culture of human gut bacteria with droplet microfluidics. *bioRxiv*, 2019: 630822.

[44] Ingham CJ, Sprenkels A, Bomer J, Molenaar D, Van Den Berg A, Van Hylckama Vlieg JET, De Vos WM. The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms. *Proceedings of the National Academy of Sciences of the United States of America*, 2007, 104(46): 18217–18222.

[45] Zengler K, Toledo G, Rappé M, Elkins J, Mathur EJ, Short JM, Keller M. Cultivating the uncultured. *Proceedings of the National Academy of Sciences of the United States of America*, 2002, 99(24): 15681–15686.

[46] Qiu YL, Hanada S, Ohashi A, Harada H, Kamagata Y, Sekiguchi Y. *Syntrophorhabdus aromaticivorans* gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. *Applied and Environmental Microbiology*, 2008, 74(7): 2051–2058.

[47] Sakai S, Imachi H, Hanada S, Ohashi A, Harada H, Kamagata Y. *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order *Methanocellales* ord. nov. *International Journal of Systematic and Evolutionary Microbiology,* 2008, 58(4): 929–936.

[48] Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature*, 2002, 417(6884): 63–67.

[49] Carbonero F, Oakley BB, Purdy KJ. Improving the isolation of anaerobes on solid media: the example of the fastidious *Methanosaeta*. *Journal of Microbiological Methods*, 2010, 80(2): 203–205.

[50] Ma TT, Cheng L, Zheng ZZ, Qin QS, Dai LR, Zhang H. Effects of pH on methanogenesis and methanogenic community in the cultures amended with acetate. *Acta Microbiologica Sinica*, 2014, 54(12): 1453–1461. (in Chinese)
麻婷婷, 承磊, 郑珍珍, 覃千山, 代莉蓉, 张辉. 不同 pH 缓冲液对由乙酸产甲烷菌群结构的影响. 微生物学报, 2014, 54(12): 1453–1461.

[51] Rettedal EA, Gumpert H, Sommer MOA. Cultivation-based multiplex phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria. *Nature Communications*, 2014, 5: 4714.

[52] Zehnder AJ, Wuhrmann K. Titanium (III) citrate as a nontoxic oxidation-reduction buffering system for the culture of obligate anaerobes. *Science*, 1976, 194(4270): 1165–1166.

[53] Rabus R, Widdel F. Utilization of alkylbenzenes during anaerobic growth of pure cultures of denitrifying bacteria on crude oil. *Applied and Environmental Microbiology*, 1996, 62(4): 1238–1241.

[54] Van Kessel MAHJ, Speth DR, Albertsen M, Nielsen PH, Op Den Camp HJM, Kartal B, Jetten MSM, Lücker S. Complete nitrification by a single microorganism. *Nature*, 2015, 528(7583): 555–559.

[55] Connon SA, Giovannoni SJ. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Applied and Environmental Microbiology*, 2002, 68(8): 3878–3885.

[56] Imachi H, Aoi K, Tasumi E, Saito Y, Yamanaka Y, Saito Y, Yamaguchi T, Tomaru H, Takeuchi R, Morono Y, Inagaki F, Takai K. Cultivation of methanogenic community from subseafloor sediments using a continuous-flow bioreactor. *The ISME Journal*, 2011, 5(12): 1913–1925.

[57] Imachi H, Sakai S, Lipp JS, Miyazaki M, Saito Y, Yamanaka Y, Hinrichs KU, Inagaki F, Takai K. *Pelolinea submarina* gen. nov., sp. nov., an anaerobic, filamentous bacterium of the phylum *Chloroflexi* isolated from subseafloor sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(3): 812–818.

[58] Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, Tyson GW. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. *Science*, 2015, 350(6259): 434–438.

[59] Wang YZ, Wegener G, Hou JL, Wang FP, Xiao X. Expanding anaerobic alkane metabolism in the domain of Archaea. *Nature Microbiology*, 2019, 4(4): 595–602.

[60] Borrel G, Adam PS, McKay LJ, Chen LX, Sierra-Garcia IN, Sieber CMK, Letourneur Q, Ghozlane A, Andersen GL, Li WJ, Hallam SJ, Muyzer G, De Oliveira VM, Inskeep WP, Banfield JF, Gribaldo S. Wide diversity of methane and short-chain alkane metabolisms in uncultured archaea. *Nature Microbiology*, 2019, 4(4): 603–613.

[61] Hua ZS, Wang YL, Evans PN, Qu YN, Goh KM, Rao YZ, Qi YL, Li YX, Huang MJ, Jiao JY, Chen YT, Mao YP, Shu WS, Hozzein W, Hedlund BP, Tyson GW, Zhang T, Li WJ. Insights into the ecological roles and evolution of methyl-coenzyme M reductase-containing hot spring Archaea. *Nature Communications*, 2019, 10(1): 4574.

[62] Vanwonterghem I, Evans PN, Parks DH, Jensen PD, Woodcroft BJ, Hugenholtz P, Tyson GW. Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. *Nature Microbiology*, 2016, 1: 16170.

[63] Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH, Von Bergen M, Rattei T, Bendinger B, Nielsen PH, Wagner M. Complete nitrification by *Nitrospira* bacteria. *Nature*, 2015, 528(7583): 504–509.

[64] Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, Krukenberg V, Meier DV, Richter M, Tegetmeyer HE, Riedel D, Richnow HH, Adrian L, Reemtsma T, Lechtenfeld OJ, Musat F. Thermophilic archaea activate butane via alkyl-coenzyme M formation. *Nature*, 2016, 539(7629): 396–401.

[65] Chen SC, Musat N, Lechtenfeld OJ, Paschke H, Schmidt M, Said N, Popp D, Calabrese F, Stryhanyuk H, Jaekel U, Zhu YG, Joye SB, Richnow HH, Widdel F, Musat F. Anaerobic oxidation of ethane by archaea from a marine hydrocarbon seep. *Nature*, 2019, 568(7750): 108–111.

[66] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, Trape JF, Koonin EV, La Scola B, Raoult D. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clinical Microbiology and Infection*, 2012, 18(12): 1185–1193.

[67] Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M, Matsui Y, Miyazaki M, Murata K, Saito Y, Sakai S, Song CH, Tasumi E, Yamanaka Y, Yamaguchi T, Kamagata Y, Tamaki H, Takai K. Isolation of an archaeon at the prokaryote–eukaryote interface. *Nature*, 2020, 577(7791): 519–525.

[68] Lopez-Garcia P, Moreira D. Cultured asgard archaea shed light on eukaryogenesis. *Cell*, 2020, 181(2): 232–235.

[69] Cross KL, Campbell JH, Balachandran M, Campbell AG, Cooper SJ, Griffen A, Heaton M, Joshi S, Klingeman D, Leys E, Yang ZM, Parks JM, Podar M. Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. *Nature Biotechnology*, 2019, 37(11): 1314–1321.

[70] Kaeberlein T, Lewis K, Epstein SS. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science*, 2002, 296(5570): 1127–1129.

[71] Locey KJ, Lennon JT. Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 2016, 113(21): 5970–5975.

[72] Schloss PD, Girard RA, Martin T, Edwards J, Thrash JC. Status of the archaeal and bacterial census: an update. *mBio*, 2016, 7(3): e00201–e00216.

[73] Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology*, 2014, 12(9): 635–645.

[74] Louca S, Mazel F, Doebeli M, Parfrey LW. A census-based estimate of Earth's bacterial and archaeal diversity. *PLoS Biology*, 2019, 17(2): e3000106.

[75] Zhou MJ, Yang XS. Pure Cultivation, isolation and identification of *Methanosarcina* sp. *Microbiology China*, 1983, 10(6): 241–243. (in Chinese)
周孟津, 杨秀山. 甲烷八叠球菌的纯培养、分离和鉴定. 微生物学通报, 1983, 10(6): 241–243.

[76] Qian ZS. Isolation and characteristics of *Methanobre-vibacter arboriphilus* and *Methanob-acterium formicicum*. *Acta Microbiologica Sinica*. 1984, 24(2): 105–110. (in Chinese)
钱泽澍. 嗜树木甲烷短杆菌和甲酸甲烷杆菌的分离和特性. 微生物学报, 1984, 24(2): 105–110.

[77] Zhao YZ, You AD. The isolation and the physiology of *Methanococcus mazei* C-44. *Acta Microbiologica Sinica*, 1984, 24(2): 99–104. (in Chinese)
赵一章, 尤爱达. 马氏甲烷球菌的分离及其生理性状的研究. 微生物学报, 1984, 24(2): 99–104.

[78] Zhang H, Zhao YZ. Isolation and characteristics of *Methanosarcina thermophilia* CB. *Acta Microbiologica Sinica*, 1987, 27(3): 195–200. (in Chinese)
张辉, 赵一章. 嗜热甲烷八叠球菌的分离和生理特性. 微生物学报, 1987, 27(3): 195–200.

[79] Zhao YZ, Zhang H, Boone DR, Mah RA. Isolation and characterization of a fast-growing, thermophilic *Methanobacterium Species*. *Applied and Environmental Microbiology*, 1986, 52(5): 1227–1229.

[80] 承磊, 仇天雷, 邓宇, 张辉. 我国产甲烷古菌研究回顾与展望//中国农业科学院院庆 50 周年论文集. 2007: 355–363.

[81] Cao XH, Liu XL, Dong XZ. *Alkaliphilus crotonatoxidans* sp. nov., a strictly anaerobic, crotonate-dismutating bacterium isolated from a methanogenic environment. *International Journal of Systematic and Evolutionary Microbiology*, 2003, 53(4): 971–975.

[82] Mu DS, Zhou LY, Liang QY, Chen GJ, Du ZJ. *Tichowtungia aerotolerans* gen. nov., sp. nov., a novel representative of the phylum Kiritimatiellaeota and proposal of Tichowtungiaceae fam. nov., *Tichowtungiales* ord. nov. and *Tichowtungiia* class. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2020, 70(9): 5001-5011.

[83] Liu L, Lv AP, Ming YZ, Jiao JY, Xiao M, Lin JG, Gu JD, Salam N, Li WJ. A xylan-degrading thermophilic and obligate anaerobe *Xylanivirga thermophila* gen. nov., sp. nov., isolated from an anammox dominant wastewater treatment plant, and proposal of *Xylanivirgaceae* fam. nov. *Anaerobe*, 2020, 61: 102075.

[84] Zhang X, Tu B, Dai LR, Lawson PA, Zheng ZZ, Liu LY, Deng Y, Zhang H, Cheng L. *Petroclostridium xylanilyticum* gen. nov., sp. nov., a xylan-degrading bacterium isolated from an oilfield, and reclassification of clostridial cluster III members into four novel genera in a new *Hungateiclostridiaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2018, 68(10): 3197–3211.

[85] Cheng L, Qiu TL, Yin XB, Wu XL, Hu GQ, Deng Y, Zhang H. *Methermicoccus shengliensis* gen. nov., sp. nov., a thermophilic, methylotrophic methanogen isolated from oil-production water, and proposal of *Methermicoccaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2007, 57(12): 2964–2969.

[86] Lv XM, Yang M, Dai LR, Tu B, Chang C, Huang Y, Deng Y, Lawson PA, Zhang H, Cheng L, Tang YQ. *Zhaonella*

formicivorans gen. nov., sp. nov., an anaerobic formate-utilizing bacterium isolated from Shengli oilfield, and proposal of four novel families and *Moorellales* ord. nov. in the phylum *Firmicutes*. *International Journal of Systematic and Evolutionary Microbiology*, 2020, 70(5): 3361–3373.

[87] Ma K, Liu XL, Dong XZ. *Methanosaeta harundinacea* sp. nov., a novel acetate-scavenging methanogen isolated from a UASB reactor. *International Journal of Systematic and Evolutionary Microbiology*, 2006, 56(1): 127–131.

[88] Rotaru AE, Shrestha PM, Liu FH, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin KP, Lovley DR. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. *Energy & Environmental Science*, 2014, 7(1): 408–415.

[89] Mayumi D, Mochimaru H, Tamaki H, Yamamoto K, Yoshioka H, Suzuki Y, Kamagata Y, Sakata S. Methane production from coal by a single methanogen. *Science*, 2016, 354(6309): 222–225.

[90] Ma K, Liu XL, Dong XZ. *Methanobacterium beijingense* sp. nov., a novel methanogen isolated from anaerobic digesters. *International Journal of Systematic and Evolutionary Microbiology*, 2005, 55(1): 325–329.

[91] Cheng L, Dai LR, Li X, Zhang H, Lu YH. Isolation and characterization of *Methanothermobacter crinale* sp. nov., a novel hydrogenotrophic methanogen from the Shengli oil field. *Applied and Environmental Microbiology*, 2011, 77(15): 5212–5219.

[92] Cheng L, Qiu TL, Li X, Wang WD, Deng Y, Yin XB, Zhang H. Isolation and characterization of *Methanoculleus receptaculi* sp. nov. from Shengli oil field, China. *FEMS Microbiology Letters*, 2008, 285(1): 65–71.

[93] Tian JQ, Wang YF, Dong XZ. *Methanoculleus hydrogenitrophicus* sp. nov., a methanogenic archaeon isolated from wetland soil. *International Journal of Systematic and Evolutionary Microbiology*, 2010, 60(9): 2165–2169.

[94] Zhang GS, Jiang N, Liu XL, Dong XZ. Methanogenesis from methanol at low temperatures by a novel psychrophilic methanogen, "*Methanolobus psychrophilus*" sp. nov., prevalent in Zoige wetland of the Tibetan plateau. *Applied and Environmental Microbiology*, 2008, 74(19): 6114–6120.

[95] Zeng X, Zhang XB, Jiang LJ, Alain K, Jebbar M, Shao ZZ. *Palaeococcus pacificus* sp. nov., an archaeon from deep-sea hydrothermal sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2013, 63(6): 2155–2159.

[96] Zhao WS, Zeng XP, Xiao X. *Thermococcus eurythermalis* sp. nov., a conditional piezophilic, hyperthermophilic archaeon with a wide temperature range for growth, isolated from an oil-immersed chimney in the Guaymas Basin. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(1): 30–35.

[97] An DD, Cai SC, Dong XZ. *Actinomyces ruminicola* sp. nov., isolated from cattle rumen. *International Journal of Systematic and Evolutionary Microbiology*, 2006, 56(9): 2043–2048.

[98] Chen M, Xiao X, Wang P, Zeng X, Wang FP. *Arthrobacter ardleyensis* sp. nov., isolated from Antarctic lake sediment and deep-sea sediment. *Archives of Microbiology*, 2005, 183(4): 301–305.

[99] Busse HJ. Review of the taxonomy of the genus *Arthrobacter*, emendation of the genus *Arthrobacter sensu lato*, proposal to reclassify selected species of the genus *Arthrobacter* in the novel genera *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov. and *Pseudarthrobacter* gen. nov., and emended description of *Arthrobacter roseus*. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(1): 9–37.

[100] Yan LE, Wang JJ, Chen ZR, Guan YY, Li J. *Microbacterium nanhaiense* sp. nov., an actinobacterium isolated from sea sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(10): 3697–3702.

[101] Cao JW, Birien T, Gayet N, Huang ZB, Shao ZZ, Jebbar M, Alain K. *Desulfurobacterium indicum* sp. nov., a thermophilic sulfur-reducing bacterium from the Indian Ocean. *International Journal of Systematic and Evolutionary Microbiology*, 2017, 67(6): 1665–1668.

[102] Su XL, Tian Q, Zhang J, Yuan XZ, Shi XS, Guo RB, Qiu YL. *Acetobacteroides hydrogenigenes* gen. nov., sp. nov., an anaerobic hydrogen-producing bacterium in the family *Rikenellaceae* isolated from a reed swamp. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(9): 2986–2991.

[103]Qiu YL, Kuang XZ, Shi XS, Yuan XZ, Guo RB. *Paludibacter jiangxiensis* sp. nov., a strictly anaerobic, propionate-producing bacterium isolated from rice paddy field. *Archives of Microbiology*, 2014, 196(3): 149–155.

[104]Tan HQ, Li TT, Zhu C, Zhang XQ, Wu M, Zhu XF. *Parabacteroides chartae* sp. nov., an obligately anaerobic species from wastewater of a paper mill. *International Journal of Systematic and Evolutionary Microbiology*, 2012, 62(11): 2613–2617.

[105]Wang YJ, Xu XJ, Zhou N, Sun YT, Liu C, Liu SJ, You X. *Parabacteroides acidifaciens* sp. nov., isolated from human faeces. *International Journal of Systematic and Evolutionary Microbiology*, 2019, 69(3): 761–766.

[106]Chen SY, Dong XZ. *Proteiniphilum acetatigenes* gen. nov., sp. nov., from a UASB reactor treating brewery wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2005, 55(6): 2257–2261.

[107]Jia C, Cui HC, Han YQ, Fu TY, Du R, Wang XL, Shi XC, Zhang XH. *Ancylomarina psychrotolerans* sp. nov., isolated from sediments of Fildes Peninsula and emended the description of genus *Ancylomarina*. *Antonie van Leeuwenhoek*, 2018, 111(7): 1183–1189.

[108]Fu TY, Cui HX, Jia C, Sun W, Zhou S, Han YQ, Liu LJ, Shi XC, Zhang XH. *Ancylomarina longa* sp. nov., isolated from southern Okinawa Trough sediment and emended description of the family *Marinifilaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 2020, 70(4): 2531–2536.

[109]Zhao C, Gao ZM, Qin QW, Ruan LW. *Mangroviflexus xiamenensis* gen. nov., sp. nov., a member of the family *Marinilabiliaceae* isolated from mangrove sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2012, 62(8): 1819–1824.

[110]Gao ZM, Liu X, Zhang XY, Ruan LW. *Thermophagus xiamenensis* gen. nov., sp. nov., a moderately thermophilic and strictly anaerobic bacterium isolated from hot spring sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2013, 63(1): 109–113.

[111]Zhang XB, Zeng X, Li X, Alain K, Jebbar M, Shao ZZ. *Anaeromicrobium sediminis* gen. nov., sp. nov., a fermentative bacterium isolated from deep-sea sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2017, 67(5): 1462–1467.

[112]Zhang R, Zeng Y, Wang TQ, Wang XM, Shi YY, Zou JT, Sun C, Zhang GS, Zhang WY, Wu M. *Anaerophilus nitritogenes* gen. nov., sp. nov., isolated from salt lake sediment in Xinjiang Province, China. *Antonie van Leeuwenhoek*, 2020, 113(3): 417–425.

[113]Zhang YZ, Fang MX, Zhang WW, Li TT, Wu M, Zhu XF. *Salimesophilobacter vulgaris* gen. nov., sp. nov., an anaerobic bacterium isolated from paper-mill wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2013, 63(4): 1317–1322.

[114]Li GY, Zeng X, Liu XP, Zhang XB, Shao ZZ. *Wukongibacter baidiensis* gen. nov., sp. nov., an anaerobic bacterium isolated from hydrothermal sulfides, and proposal for the reclassification of the closely related *Clostridium halophilum* and *Clostridium caminithermale* within *Maledivibacter* gen. nov. and *Paramaledivibacter* gen. nov., respectively. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(11): 4355–4361.

[115]Wu YF, Zheng H, Wu QL, Yang H, Liu SJ. *Clostridium algifaecis* sp. nov., an anaerobic bacterial species from decomposing algal scum. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(11): 3844–3848.

[116]Song L, Dong XZ. *Clostridium amylolyticum* sp. nov., isolated from H_2-producing UASB granules. *International Journal of Systematic and Evolutionary Microbiology*, 2008, 58(9): 2132–2135.

[117]Dong YY, Liu YH, Chen NC, Zhong YJ, Liu L, Xie QL. *Clostridium beihaiense* sp. nov., an anaerobic bacterium isolated from activated sludge. *International Journal of Systematic and Evolutionary Microbiology*, 2018, 68(9): 2789–2793.

[118] Zhu H, Fu B, Lu ST, Liu HB, Liu H. *Clostridium bovifaecis* sp. nov., a novel acetogenic bacterium isolated from cow manure. *International Journal of Systematic and Evolutionary Microbiology*, 2018, 68(9): 2956–2959.

[119] Xu PX, Chai LJ, Qiu T, Zhang XJ, Lu ZM, Xiao C, Wang ST, Shen CH, Shi JS, Xu ZH. *Clostridium fermenticellae* sp. nov., isolated from the mud in a fermentation cellar for the production of the Chinese liquor, Baijiu. *International Journal of Systematic and Evolutionary Microbiology*, 2019, 69(3): 859–865.

[120]Zhao X, Li DY, Xu SH, Guo ZH, Zhang Y, Man L, Jiang BH, Hu XM. *Clostridium guangxiense* sp. nov. and *Clostridium neuense* sp. nov., two phylogenetically closely related hydrogen-producing species isolated from lake sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2017, 67(3): 710–715.

[121]Ruan ZY, Wang YW, Zhang C, Song JL, Zhai Y, Zhuang Y, Wang HM, Chen XR, Li YT, Zhao BQ, Zhao B. *Clostridium huakuii* sp. nov., an anaerobic, acetogenic bacterium isolated from methanogenic consortia. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(12): 4027–4032.

[122]Yin Q, Tao Y, Zhu XY, Zhou Y, He XH, Cheng L, Huang Y, Li DP. *Clostridium liquoris* sp. nov., isolated from a fermentation pit used for the production of Chinese strong-flavoured liquor. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(2): 749–754.

[123]Wang Q, Wang CD, Li CH, Li JG, Chen Q, Li YZ. *Clostridium luticellarii* sp. nov., isolated from a mud cellar used for producing strong aromatic liquors. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(12): 4730–4733.

[124]Huang Y, Wei ZX, Cong LL, Qiu ZW, Chen R, Deng Y, Zhang YG, Fan H, Ma SC. *Clostridium prolinivorans* sp. nov., a thermophilic bacterium isolated from an anaerobic reactor degrading propionate. *International Journal of Systematic and Evolutionary Microbiology*, 2019, 70(10): 5190–5196.

[125]Liu CL, Huang D, Liu LY, Zhang J, Deng Y, Chen L, Zhang WX, Wu ZY, Fan A, Lai DY, Dai LR. *Clostridium swellfunianum* sp. nov., a novel anaerobic bacterium isolated from the pit mud of Chinese *Luzhou-flavor* liquor production. *Antonie van Leeuwenhoek*, 2014, 106(4): 817–825.

[126] Wu D, Zhang NF, Sun C, Zhang WW, Han SB, Pan J, Wu M, Th D, Zhu XF. *Haloimpatiens lingqiaonensis* gen. nov., sp. nov., an anaerobic bacterium isolated from paper-mill wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(2): 628–632.

[127]Pi RX, Zhang WW, Fang MX, Zhang YZ, Li TT, Wu M, Zhu XF. *Oceanirhabdus sediminicola* gen. nov., sp. nov., an anaerobic bacterium isolated from sea sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2013, 63(11): 4277–4283.

[128]Zhang KG, Song L, Dong XZ. *Proteiniclasticum ruminis* gen. nov., sp. nov., a strictly anaerobic proteolytic bacterium isolated from yak rumen. *International Journal of Systematic and Evolutionary Microbiology*, 2010, 60(9): 2221–2225.

[129]Ji SQ, Wang B, Lu M, Li FL. *Defluviitalea phaphyphila* sp. nov., a novel thermophilic bacterium that degrades brown algae. *Applied and Environmental Microbiology*, 2016, 82(3): 868–877.

[130]Ma SC, Huang Y, Wang C, Fan H, Dai LR, Zhou Z, Liu X, Deng Y. *Defluviitalea raffinosedens* sp. nov., a thermophilic, anaerobic, saccharolytic bacterium isolated from an anaerobic batch digester treating animal manure and rice straw. *International Journal of Systematic and Evolutionary Microbiology*, 2017, 67(5): 1607–1612.

[131]Cai SC, Dong XZ. *Cellulosilyticum ruminicola* gen. nov., sp. nov., isolated from the rumen of yak, and reclassification of *Clostridium lentocellum* as *Cellulosilyticum lentocellum* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2010, 60(4): 845–849.

[132]Wu XY, Shi KL, Xu XW, Wu M, Oren A, Zhu XF. *Alkaliphilus halophilus* sp. nov., a strictly anaerobic and halophilic bacterium isolated from a saline lake, and emended description of the genus *Alkaliphilus*. *International Journal of Systematic and Evolutionary Microbiology*, 2010, 60(12): 2898–2902.

[133]Chen SY, Dong XZ. *Acetanaerobacterium elongatum* gen. nov., sp. nov., from paper mill waste water. *International Journal of Systematic and Evolutionary Microbiology*, 2004, 54(6): 2257–2262.

[134]Xing DF, Ren NQ, Li QB, Lin M, Wang AJ, Zhao LH. *Ethanoligenens harbinense* gen. nov., sp. nov., isolated from molasses wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2006, 56(4): 755–760.

[135]Song L, Dong XZ. *Hydrogenoanaerobacterium saccharovorans* gen. nov., sp. nov., isolated from H_2-producing UASB granules. *International Journal of Systematic and Evolutionary Microbiology*, 2009, 59(2): 295–299.

[136]Chen SY, Niu LL, Zhang YX. *Saccharofermentans acetigenes* gen. nov., sp. nov., an anaerobic bacterium

isolated from sludge treating brewery wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2010, 60(12): 2735–2738.

[137]Yang GQ, Guo JH, Zhuang L, Yuan Y, Zhou SG. *Desulfotomaculum ferrireducens* sp. nov., a moderately thermophilic sulfate-reducing and dissimilatory Fe(III)-reducing bacterium isolated from compost. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(8): 3022–3028.

[138]Wang YW, Song JL, Zhai Y, Zhang C, Gerritsen J, Wang HM, Chen XR, Li YT, Zhao BQ, Zhao B, Ruan ZY. *Romboutsia sedimentorum* sp. nov., isolated from an alkaline-saline lake sediment and emended description of the genus *Romboutsia*. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(4): 1193–1198.

[139]Chen SY, Song L, Dong XZ. *Sporacetigenium mesophilum* gen. nov., sp. nov., isolated from an anaerobic digester treating municipal solid waste and sewage. *International Journal of Systematic and Evolutionary Microbiology*, 2006, 56(4): 721–725.

[140]Deng Y, Guo X, Wang YW, He MX, Ma KD, Wang HM, Chen XR, Kong DL, Yang ZR, Ruan ZY. *Terrisporobacter petrolearius* sp. nov., isolated from an oilfield petroleum reservoir. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(10): 3522–3526.

[141]Wu CG, Liu XL, Dong XZ. *Syntrophomonas cellicola* sp. nov., a spore-forming syntrophic bacterium isolated from a distilled-spirit-fermenting cellar, and assignment of *Syntrophospora bryantii* to *Syntrophomonas bryantii* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2006, 56(10): 2331–2335.

[142]Zhang CY, Liu XL, Dong XZ. *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens. *International Journal of Systematic and Evolutionary Microbiology*, 2004, 54(3): 969–973.

[143]Zhang CY, Liu XL, Dong XZ. *Syntrophomonas erecta* sp. nov., a novel anaerobe that syntrophically degrades short-chain fatty acids. *International Journal of Systematic and Evolutionary Microbiology*, 2005, 55(2): 799–803.

[144]Zhang F, Liu XL, Dong XZ. *Thermosyntropha tengcongensis* sp. nov., a thermophilic bacterium that degrades long-chain fatty acids syntrophically. *International Journal of Systematic and Evolutionary Microbiology*, 2012, 62(4): 759–763.

[145]Fang MX, Zhang WW, Zhang YZ, Tan HQ, Zhang XQ, Wu M, Zhu XF. *Brassicibacter mesophilus* gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2012, 62(12): 3018–3023.

[146]Wang B, Ji SQ, Tian XX, Qu LY, Li FL. *Brassicibacter thermophilus* sp. nov., a thermophilic bacterium isolated from coastal sediment. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(9): 2870–2874.

[147]Zeng X, Zhang Z, Li X, Jebbar M, Alain K, Shao ZZ. *Caloranaerobacter ferrireducens* sp. nov., an anaerobic, thermophilic, iron (III)-reducing bacterium isolated from deep-sea hydrothermal sulfide deposits. *International Journal of Systematic and Evolutionary Microbiology*, 2015, 65(6): 1714–1718.

[148]Niu LL, Song L, Dong XZ. *Proteiniborus ethanoligenes* gen. nov., sp. nov., an anaerobic protein-utilizing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 2008, 58(1): 12–16.

[149]Sun YT, Zhou N, Wang BJ, Liu XD, Jiang CY, Ge X, Liu SJ. *Vallitalea okinawensis* sp. nov., isolated from Okinawa Trough sediment and emended description of the genus *Vallitalea*. *International Journal of Systematic and Evolutionary Microbiology*, 2019, 69(2): 404–410.

[150]Chen XJ, Wang ZQ, Zhou ZY, Zeng NY, Huang QF, Wang ZW, Tang WL, Zhou HW. Characterization of *Peptacetobacter hominis* gen. nov., sp. nov., isolated from human faeces, and proposal for the reclassification of *Clostridium hiranonis* within the genus *Peptacetobacter*. *International Journal of Systematic and Evolutionary Microbiology*, 2020, 70(5): 2988–2997.

[151]Liu L, Jiao JY, Salam N, Zhou EM, Fang BZ, Xian WD, Li MM, Ding YP, Li WJ. *Rhabdanaerobium thermarum* gen. nov., sp. nov., a novel anaerobic bacterium isolated from a hot spring. *International Journal of Systematic and Evolutionary Microbiology*, 2017, 67(11): 4584–4588.

[152]Zeng X, Zhang Z, Li X, Zhang XB, Cao JW, Jebbar M, Alain K, Shao ZZ. *Anoxybacter fermentans* gen. nov., sp. nov., a piezophilic, thermophilic, anaerobic, fermentative bacterium isolated from a deep-sea hydrothermal vent. *International*

Journal of Systematic and Evolutionary Microbiology, 2015, 65(2): 710–715.

[153] Shang SM, Qian L, Zhang X, Li KZ, Chagan I. *Themoanaerobacterium calidifontis* sp. nov., a novel anaerobic, thermophilic, ethanol-producing bacterium from hot springs in China. *Archives of Microbiology*, 2013, 195(6): 439–445.

[154] Zhang X, Tu B, Lv XM, Lawson PA, Yang M, Dai LR, Zhang H, Tang YQ, Deng Y, Cheng L. Description of *Biomaibacter acetigenes* gen. nov., sp. nov., and proposal of *Thermosediminibacterales* ord. nov. containing two novel families of *Tepidanaerobacteraceae* fam. nov. and *Thermosediminibacteraceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2019, 69(12): 3891–3902.

[155] Liu Y, Qiao JT, Yuan XZ, Guo RB, Qiu YL. *Hydrogenispora ethanolica* gen. nov., sp. nov., an anaerobic carbohydrate-fermenting bacterium from anaerobic sludge. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(5): 1756–1762.

[156] Zhang KG, Dong XZ. *Selenomonas bovis* sp. nov., isolated from yak rumen contents. *International Journal of Systematic and Evolutionary Microbiology*, 2009, 59(8): 2080–2083.

[157] Zhang WW, Fang MX, Tan HQ, Zhang XQ, Wu M, Zhu XF. *Pectinatus brassicae* sp. nov., a Gram-negative, anaerobic bacterium isolated from salty wastewater. *International Journal of Systematic and Evolutionary Microbiology*, 2012, 62(9): 2145–2149.

[158] Wu KJ, Dai LR, Tu B, Zhang X, Zhang H, Deng Y, Lawson PA, Cheng L. *Gudongella oleilytica* gen. nov., sp. nov., an aerotorelant bacterium isolated from Shengli oilfield and validation of family *Tissierellaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 2020, 70(2): 951–957.

[159] Huang Y, Sun YJ, Ma SC, Chen L, Zhang H, Deng Y. Isolation and characterization of *Keratinibaculum paraultunense* gen. nov., sp. nov., a novel thermophilic, anaerobic bacterium with keratinolytic activity. *FEMS Microbiology Letters*, 2013, 345(1): 56–63.

[160] Niu LL, Song L, Liu XL, Dong XZ. *Tepidimicrobium xylanilyticum* sp. nov., an anaerobic xylanolytic bacterium, and emended description of the genus *Tepidimicrobium*. *International Journal of Systematic and Evolutionary Microbiology*, 2009, 59(11): 2698–2701.

[161] Woo PCY, Teng JLL, Leung KW, Lau SKP, Wong MKM, Yuen KY. Bacteremia in a patient with colonic carcinoma caused by a novel *Sedimentibacter* species: *Sedimentibacter hongkongensis* sp. nov. *Diagnostic Microbiology and Infectious Disease*, 2004, 50(2): 81–87.

[162] Liu DY, Liu Y, Men XH, Guo QQ, Guo RB, Qiu YL. Isolation and characterization of *Thermopirellula anaerolimosa* gen. nov., sp. nov., an obligate anaerobic hydrogen-producing bacterium of the phylum *Planctomycetes*. *Acta Microbiologica Sinica*, 2012, 52(8): 994–1001. (in Chinese)
刘冬英, 刘奕, 门学慧, 郭群群, 郭荣波, 邱艳玲. 浮霉菌门严格厌氧产氢细菌(*Thermopirellula anaerolimosa*)的分离及其生理特性. 微生物学报, 2012, 52(8): 994–1001.

[163] Sun D, Wang AJ, Cheng SA, Yates M, Logan BE. *Geobacter anodireducens* sp. nov., an exoelectrogenic microbe in bioelectrochemical systems. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(10): 3485–3491.

[164] Zhou SG, Yang GQ, Lu Q, Wu M. *Geobacter soli* sp. nov., a dissimilatory Fe(III)-reducing bacterium isolated from forest soil. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(11): 3786–3791.

[165] Cao JW, Gayet N, Zeng X, Shao ZZ, Jebbar M, Alain K. *Pseudodesulfovibrio indicus* gen. nov., sp. nov., a piezophilic sulfate-reducing bacterium from the Indian Ocean and reclassification of four species of the genus *Desulfovibrio*. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(10): 3904–3911.

[166] Chen SY, Liu XL, Dong XZ. *Syntrophobacter sulfatireducens* sp. nov., a novel syntrophic, propionate-oxidizing bacterium isolated from UASB reactors. *International Journal of Systematic and Evolutionary Microbiology*, 2005, 55(3): 1319–1324.

[167] Cai L, Shao MF, Zhang T. Non-contiguous finished genome sequence and description of *Sulfurimonas hongkongensis* sp. nov., a strictly anaerobic denitrifying, hydrogen- and sulfur-oxidizing chemolithoautotroph isolated from marine sediment. *Standards in Genomic Sciences*, 2014, 9(3): 1302–1310.

[168]Xu MY, Guo J, Cen YH, Zhong XY, Cao W, Sun GP. *Shewanella decolorationis* sp. nov., a dye-decolorizing bacterium isolated from activated sludge of a waste-water treatment plant. *International Journal of Systematic and Evolutionary Microbiology*, 2005, 55(1): 363–368.

[169]Qiu YL, Hanada S, Kamagata Y, Guo RB, Sekiguchi Y. *Lactivibrio alcoholicus* gen. nov., sp. nov., an anaerobic, mesophilic, lactate-, alcohol-, carbohydrate- and amino-acid-degrading bacterium in the phylum *Synergistetes*. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(6): 2137–2145.

[170]Lai QL, Cao JW, Dupont S, Shao ZZ, Jebbar M, Alain K. *Thermodesulfatator autotrophicus* sp. nov., a thermophilic sulfate-reducing bacterium from the Indian Ocean. *International Journal of Systematic and Evolutionary Microbiology*, 2016, 66(10): 3978–3982.

[171]Feng YX, Cheng L, Zhang XX, Li X, Deng Y, Zhang H. *Thermococcoides shengliensis* gen. nov., sp. nov., a new member of the order *Thermotogales* isolated from oil-production fluid. *International Journal of Systematic and Evolutionary Microbiology*, 2010, 60(4): 932–937.

[172]Nunoura T, Hirai M, Imachi H, Miyazaki M, Makita H, Hirayama H, Furushima Y, Yamamoto H, Takai K. *Kosmotoga arenicorallina* sp. nov. a thermophilic and obligately anaerobic heterotroph isolated from a shallow hydrothermal system occurring within a coral reef, southern part of the Yaeyama Archipelago, Japan, reclassification of *Thermococcoides shengliensis* as *Kosmotoga shengliensis* comb. nov., and emended description of the genus *Kosmotoga*. *Archives of Microbiology*, 2010, 192(10): 811–819.

[173]L'Haridon S, Jiang LJ, Alain K, Chalopin M, Rouxel O, Beauverger M, Xu HX, Shao ZZ, Jebbar M. *Kosmotoga pacifica* sp. nov., a thermophilic chemoorganoheterotrophic bacterium isolated from an East Pacific hydrothermal sediment. *Extremophiles*, 2014, 18(1): 81–88.

[174]Cai JG, Wang YP, Liu DB, Zeng Y, Xue YF, Ma YH, Feng Y. *Fervidobacterium changbaicum* sp. nov., a novel thermophilic anaerobic bacterium isolated from a hot spring of the Changbai Mountains, China. *International Journal of Systematic and Evolutionary Microbiology*, 2007, 57(10): 2333–2336.

[175]Qiu YL, Kuang XZ, Shi XS, Yuan XZ, Guo RB. *Terrimicrobium sacchariphilum* gen. nov., sp. nov., an anaerobic bacterium of the class *'Spartobacteria'* in the phylum *Verrucomicrobia*, isolated from a rice paddy field. *International Journal of Systematic and Evolutionary Microbiology*, 2014, 64(5): 1718–1723.

[176]Musat N, Musat F, Weber PK, Pett-Ridge J. Tracking microbial interactions with NanoSIMS. *Current Opinion in Biotechnology*, 2016, 41: 114–121.

[177]Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T. Insights into the phylogeny and coding potential of microbial dark matter. *Nature*, 2013, 499(7459): 431–437.

Cultivation and isolation of Anaerobes: past, present and future

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Abstract: Anaerobes are the largest number of microorganisms on earth but rarely reported from the point view of taxonomy. Owing to oxygen-sensitive and slow-growth, cultivation and isolation of fastidious anaerobe faces a great challenge. In this review, we introduce the research history of anaerobes, and analyze the factors affecting the isolating efficiency of anaerobes. Furthermore, we discuss the current status of isolation and cultivation methods and strategies, and summarize the research progress on systematic taxonomy of anaerobes. In the end, we give an outlook for isolating and cultivating anaerobes.

Keywords: anaerobes, isolation and cultivation, high-throughput screening, targeted isolation

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承磊，博士，研究员/博导，主要从事厌氧微生物资源与利用研究。研究方向 1：厌氧微生物分离、鉴定、保藏、分类和功能评价研究。提出产甲烷古菌等多个厌氧微生物新科新目，开发了厌氧微生物长期保藏技术和厌氧微生物高通量筛选平台，建立并负责国内最大、国际第四的厌氧微生物模式物种库——中国厌氧微生物资源管理中心，可以提供厌氧微生物新菌株的保藏证明。研究方向 2：石油烃厌氧生物降解产甲烷机理研究。构建了多个原油生物气化菌系模型，发现了长链烷基烃优先降解的独特地化特征和微生物机理，阐明了原油生物气化过程中的群落演替、降解机理和环境胁迫应答机制。主持国家自然科学基金等 6 项国家级课题，发表研究论文 30 余篇(https://scholar.google.com/citations?user=DZEhwUoAAAAJ&hl=zh-CN&oi=ao)。