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评 述

基于基因组信息指导的古菌培养新策略

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摘要 古菌、细菌及真核生物共同组成了地球上的三域生命体系. 其中古菌域不仅包含了现今地球上最古老的生命类群, 如产甲烷古菌; 同时, 阿斯加德(Asgard)古菌超门还被认为是真核生物的共同祖先. 近些年来, 随着地球科学和生命科学交叉研究日益加深, 科学家发现古菌在地球化学元素循环中也起到了显著作用. 通过不依赖于纯培养的高通量测序技术分析, 表明古菌具有丰富的物种多样性, 现已发现超过20个全新的古菌门. 然而, 目前仅在其中4个古菌门中获得了纯培养菌株, 对古菌生理特征、起源与演化以及生态功能的认识也受到纯培养古菌菌株稀缺的限制, 无法通过实验验证环境中未培养古菌基因组所预测到的功能基因. 在本综述中, 简述了古菌的发现与研究进展, 对比了未培养和纯培养古菌之间的差距, 并呼吁将更多的努力和科研资源投入到古菌分离和纯培养工作中. 文章还概述了基于大量未培养古菌基因组指导分离和纯培养的新思路和新策略, 即: 基因组功能预测、代谢互作网络构建、基因组水平代谢建模、机器学习预测培养条件, 期望能够使用现代高通量测序技术所积累的未培养古菌基因组数据来帮助获得更多新的纯培养古菌菌株.

关键词 古菌, 富集培养, 高通量测序, 未培养古菌, 基因组, 代谢网络

1 引言

自Carl R. Woese开创性的将古菌列为生命三域之一以来, 古菌领域的研究已有40余年, 虽然在少数模式菌株中获得了重要突破, 但是对古菌整体的认识仍相对较少(Woese和Fox, 1977; Woese等, 1990; Spang等, 2017; Baker等, 2020). 古菌最初被认为是细菌域中数个特殊分支, 主要生活在高温和酸碱性等极端环境中

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(Stetter, 2006). 1990年, Woese等通过对该类微生物核糖体小亚基(16S rRNA)基因序列的研究, 提出将其划分到一个新的生命域, 即古菌域(原名古细菌域), 与细菌域和真核生物域并列, 改变了传统的二域生命学说(Woese等, 1990). 目前, 地球生命的三域分类逐渐被科学家接受, 直到最近几年, 更多的研究证据显示真核生物可能起源于一个古菌超门, Asgard(阿斯加德, 北欧神话中神族的殿堂)支持了经典的"eocyte"假说, 即认为真核生物与泉古菌有更近的进化关系(Lake等, 1984), 形成了地球生命由细菌和古菌所组成的二域分类形式(Spang等, 2015; Zaremba-Niedzwiedzka等, 2017; Spang等, 2017; Nagrale和Gawande, 2018; Imachi等, 2020).

生命起源之后, 古菌与固体地球表层环境紧密相互作用并协同演化. 一方面, 古菌可以改变地球表面环境, 例如产甲烷古菌可以通过小分子代谢过程产生大量甲烷释放到大气中, 而甲烷是一种极强的温室气体, 推测该过程可以改变地球的气候(Battistuzzi等, 2004; Ueno等, 2006; Wolfe和Fournier, 2018); 另一方面, 古菌同时受到环境变化的胁迫, 能够通过自身进化以适应新的环境, 而相关的进化过程信息会被记录在古菌的基因组中, 例如大氧化事件(Great Oxygenation Event, GOE)可能促进了奇古菌门(Thumarchaeota)中有氧氨氧化代谢特征的起源(Ren等, 2019).

在现代环境中, 古菌在地球各环境中分布广泛(Schleper等, 2005; Wu等, 2009; Spang等, 2017). 其不仅在极端环境中被发现, 如热泉(Whitaker等, 2003)、热液喷口(Takai和Nakamura, 2011)、酸性矿水(Edwards等, 2000; Kuang等, 2016)、动物消化系统(Janssen和Kirs, 2008), 而且在全球的土壤、海洋和淡水及沉积物(Auguet等, 2010; Offre等, 2013; Mendes等, 2013)、空气(Amo等, 2002)、深部生物圈(Biddle等, 2006; Hoshino和Inagaki, 2018)以及人体(Probst等, 2013; Koskinen等, 2017)都有发现. 最近10年, 由于高通量测序技术迅速发展, 特别是(宏)基因组学的应用, 科学家在更多的生态环境中发现了种类繁多且代谢特征独特的古菌, 极大的拓展了人们对古菌的认知(Handelsman, 2004; Bragg和Tyson, 2014; Hug等, 2016; Wang和Jia, 2016; Parks等, 2017; Wang等, 2019b). 然而, 与大量基于(宏)基因组的研究相比, 分离和纯培养古菌模式菌株的进展甚微. 由于古菌模式菌株的缺乏, 阻碍了对不同门类古菌遗传操纵系统的建立, 限制了科学家对古菌代谢特征和生态功能方面的深入认知. 本综述主要阐述了如何使用目前已获得的大量古菌基因组信息来指导古菌模式菌株分离和纯培养.

2 传统古菌分离及纯培养方法概述

分离和纯培养方法在微生物学的研究中已经沿用了百余年, 是了解微生物代谢特征最直接的方法. 古菌和细菌的传统分离及纯培养方法较为类似(Huber等, 1995, 2000; Nichols, 2007; Narihiro和Kamagata, 2013), 主要步骤都包括: 采集天然样品并尽量减少外源污染, 测量原位环境的理化参数, 获得样品中微生物多样性信息, 通过荧光原位杂交(Fluorescence *in situ* hybridization, FISH)或其他有效的杂交技术识别目标细胞(DeLong等, 1989). 选择合适的培养基和生长条件, 然后接种样品, 在液体培养基中连续稀释或涂布到固体培养基上. 在此过程中, 特定培养基和生长条件是筛选微生物的关键因素, 通常可以通过对文献及数据库进行全面搜索从而仔细选择合适的培养组分. 其中包括专业期刊, 如*International Journal of Systematic and Evolutionary Microbiology*, 特定主题的书籍或数据库, 如*The Prokaryotes* (Springer)和*Bergey's Manual of Systematics of Archaea and Bacteria* (John Wiley & Sons, Inc.), 以及不同国家的微生物菌种保藏中心, 如美国模式培养物保藏中心(the American Type Culture Collection, ATCC)、德国微生物菌种保藏中心(the Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ)、中国普通微生物菌种保藏管理中心(the China General Microbiological Culture Collection Center, CGMCC), 以及日本微生物培养物保藏中心(the Japanese Culture Collection of Microorganisms, JCM)等. 最后通过挑取在固体平板上生长的单菌落进行反复涂布, 或通过极限稀释到单个细胞从而获取液体培养. 再以适当的方式, 如光学显微镜或电子显微镜, 以及16S rRNA基因克隆文库, 验证培养物的纯度. 成功使用上述方法实现微生物分离及纯培养的研究还可以参考Tamaki(2019)、Sun等(2020)和Salam等(2021)综述.

此外, 分离特殊环境的微生物最重要的是建立适当的生长条件. 例如, 目前绝大多数微生物模式菌株

都是在正常大气压力下进行分离和纯培养，然而在深部生物圈的高压环境下生活着大量不同类型的嗜压微生物(Kallmeyer等, 2012; Inagaki等, 2015; Zhang Y等, 2015). 自20世纪40年代以来，研究人员已经意识到嗜压微生物对压力变化非常敏感, 在样品采集过程中, 其很可能在从深海或深部环境到海面或地面的运输时, 因为压力变化而失去活性(Demazeau和Rivalain, 2011). 因此，在采集和培养过程中都需要高静水压(High hydrostatic pressure, HHP)环境. 目前的高压培养技术能够以小于1毫升到数十升的体积，批式或连续的，甚至以气体作为底物进行微生物富集培养(Parkes等, 2009; Zhang Y等, 2015). 使用批式高压培养已分离得到的嗜压古菌包括*Methanocaldococcus*、*Methanopyrus*、*Palaeococcus*、*Thermococcus*和*Pyrococcus*等(Boonyaratanakornkit等, 2007; Takai等, 2008; Zeng等, 2009, 2013; Birrien等, 2011; Vannier等, 2011; Zhang Y等, 2015)，以及通过连续流动气液高压系统，再经过数年富集培养出甲烷厌氧氧化古菌(ANME)-2a类群(Wang等, 2014). 最近，从太平洋海床下深煤层2.5km处和Nankai海槽增生杂岩体等天然高压环境中也分离出了产甲烷古菌(Inagaki等, 2015; Ijiri等, 2018).

通过使用传统方法, 在过去数十年中已有4个古菌门中超过10个纲的古菌菌株被分离和纯培养(图1). 这些菌株大多来自广古菌门(Euryarchaeota)，其中包括Archaeoglobi纲(Brileya和Reysenbach, 2014)、Halobacteria纲(Oren, 2006)、Thermococci纲(Zhao等, 2015; Zillig和Reysenbach, 2015)、Thermoplasmata纲(Reysenbach, 2015a)、Methanobacteria纲(Boone, 2015a)、Methanococci纲(Boone, 2015b)、Methanopyri纲(Garrity和Holt, 2015)、Methanomicrobia纲(Garcia等, 2006; Kendall和Boone, 2006)，以及Methanonatronarchaeia纲(Sorokin等, 2018). 其余纯培养的古菌菌株还包括泉古菌门(Crenarchaeota)中的Thermoprotei纲(Reysenbach, 2015b)以及奇古菌门和纳古菌门(Nanoarchaeota)中的数个菌株(Könneke等, 2005; Huber等, 2006; Brochier-Armanet等, 2008; Wurch等, 2016). 然而，大部分这些门/纲水平的菌株都是在10年前被分离和报道，在过去10年内，仅有少数新的古菌模式菌株通过传统方法获得其分离培养物，例如在广古菌门的Methanonatronarchaeia纲和Thermoplasmata纲的Methanomassiliicoccales目(Dridi等, 2012; Nkamga和Drancourt, 2015; Sorokin等, 2017). 古菌分离和纯培养进展缓慢的原因较多, 其中之一是传统培养方法已经达到瓶颈, 即利用常见组分确定的培养基可以培养的古菌种类已经达到

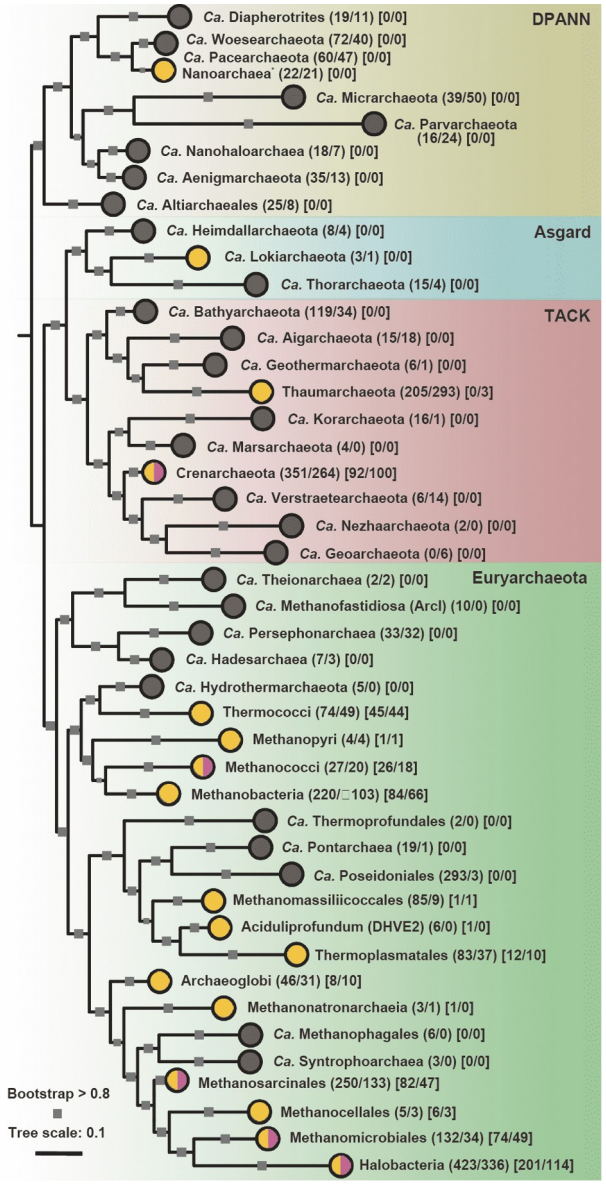

图 1　通过使用37个串联保守蛋白质序列构建的古菌域系统发育树

发育树共涵盖了所有4个古菌超门，即DPANN、Asgard、TACK和Euryarchaeota，不同背景颜色代表不同古菌超门. 圆圈代表不同古菌类群，其中灰色代表仅有基因组，但没有纯培养菌株或基因组水平代谢模型(Genome-scale metabolic models, GEM); 黄色代表有基因组及纯培养菌株. 黄色加粉色表示同时包含基因组、纯培养菌株和GEM模型. (*x*/*y*)中的数字表示该古菌类群数据库中基因组的数目(NCBI/IMG)，而[*x*/*y*]表示该古菌类群中可以纯培养模式菌株的数目[DSMZ/GOLD]

饱和. 与此同时, 目前使用不依赖于培养的高通量测序技术以及环境地球化学方法等代替分离和纯培养方法, 成为古菌研究领域的新热点, 许多研究人员和资金资源已转向该新方向, 这也导致了古菌分离和纯培养的研究进展相对滞后.

3 高通量测序技术拓展古菌基因组多样性

目前常用的高通量测序方法包括使用新一代测序平台, 如Illumina HiSeq、Oxford Nanopore等测序仪, 对16S rRNA基因或功能基因进行扩增子测序, 以及对环境总DNA或RNA进行宏基因组或宏转录组测序(Faust等, 2015; Quince等, 2017), 其中可能包含样品中几乎所有微生物的基因组信息(Handelsman, 2004; Bragg和Tyson, 2014; Cowan等, 2015). 这些分析最初是用于预测环境中总体微生物组成和代谢潜力. 例如, 通过宏基因组和宏转录组方法研究了瓜伊马斯盆地(Guaymas Basin)热液区沉积物的微生物代谢情况, 并发现碳代谢和硫代谢基因在原位环境中高度活跃表达, 其中Archaeoglobi纲的古菌以及Deltaproteobacteria和Epsilonproteobacteria纲的细菌占主导地位(He等, 2013). 此外, Tyson等(2004)提出了一种基于不同微生物基因组特征提取单一物种基因组的方法, 这种方法被称为宏基因组拼接基因组(Metagenome-assembled genome, MAG). 该方法通过使用DNA序列信息的算法来对宏基因组中的DNA序列进行分箱(binning), 并很快成为环境微生物研究中广泛使用的分析程序(Albertsen等, 2013; Anantharaman等, 2016; Hug等, 2016; Parks等, 2017). 最近, 随着MAG评估工具和MAG质量标准的提出, 进一步推动了该方法的普及使用与认可度(Parks等, 2015; Bowers等, 2017). 例如, 一项研究通过对冰川冻土碳循环过程中214个样品进行宏基因组测序并获得了1529个MAGs, 从而较为全面的描述了冰川冻土生态系统中微生物对碳循环过程的影响和驱动(Woodcroft等, 2018).

通过高通量测序及宏基因组学分析方法, 我们对古菌代谢的认知有了极大的扩展(Spang等, 2017; Baker等, 2020). 在过去15年中, 古菌域的新成员从两个门(泉古菌门和广古菌门)增加到目前包含基因组信息的超过20个门(图1). 这些新的古菌类群具有独特的代谢特征, 并且广泛分布于全球不同生态系统中, 如土壤、沉积物、海洋湖泊、生物反应器或酸性矿水、甚至深部生物圈等(Hug等, 2016; Parks等, 2017; Spang等, 2017).

在广古菌门中, 新发现的古菌物种包括甲烷厌氧氧化古菌(Wu等, 2009; Meyerdierks等, 2010; Haroon等, 2013; Wang等, 2014; Krukenberg等, 2018; Wang等, 2019a), 多碳烷烃代谢古菌*Candidatus* (*Ca.*) Syntrophoarchaeum spp.、*Ca.* Argoarchaeum spp.和*Ca.* Ethanoperedens spp.(Laso-Pérez等, 2016; Chen等, 2019; Hahn等, 2020), 产甲烷古菌*Ca.* Methanofastidiosa (Nobu等, 2016), *Ca.* Methanoliparia(Borrel等, 2019; Laso-Pérez等, 2019), 潜在的多碳烷烃代谢型Archaeoglobi(Boyd等, 2019; Wang等, 2019b)和硫酸盐还原甲烷代谢型Archaeoglobi(Colman等, 2019; Wang等, 2019b). 另外还包括异养古菌*Ca.* Thalassoarchaea (MG-II)和*Ca.* Pontarchaea(MG-III)(Iverson等, 2012; Li等, 2015; Zhang C L等, 2015; Xie等, 2018), *Ca.* Thermoprofundales(MBGD archaea)(Lloyd等, 2013; Lazar等, 2017; Zhou等, 2019), *Ca.* Hadesarchaeota(Baker等, 2016), *Ca.* Persephonarchaea(MSBL-1, Mwirichia等, 2016), 以及*Ca.* Theionarchaea(Lazar等, 2017)和*Ca.* Hydrothermarchaeota(Carr等, 2019; Zhou等, 2020), 还有最近报道的新的极端嗜盐古菌Halobacteria(Becker等, 2014; Sorokin等, 2016, 2017).

在TACK超门, 除了奇古菌门中独特的自养氨氧化古菌(Brochier-Armanet等, 2008; Pester等, 2011), 其余大部分可以进行有机质代谢, 并包含Wood-Ljungdahl(WL)通路, 暗示它们能够通过有机底物和CO_2产生乙酰辅酶A. 新发现的TACK超门中还包括深古菌门*Ca.* Bathyarchaeota(He等, 2016; Zhou等, 2018), *Ca.* Geoarchaeota(Kozubal等, 2013)和*Ca.* Aigarchaeota(Hedlund等, 2015; Hua等, 2018). 最近还发现了*Ca.* Geothermarchaeota(Jungbluth等, 2017)、*Ca.* Marsarchaeota(Jay等, 2018), 以及新的产甲烷古菌*Ca.* Verstraetearchaeota(Vanwonterghem等, 2016; Berghuis等, 2019), *Ca.* Nezhaarchaeota(Wang等, 2019b)和*Ca.* Korarchaeota(McKay等, 2019; Wang等, 2019b).

在Asgard超门中(Williams等, 2013), 通过基因组证据表明, 新发现的*Ca.* Lokiarchaeota(Nasir等, 2015; Sousa等, 2016; Orsi等, 2019; Imachi等, 2020)、*Ca.* Thorarchaeota(Seitz等, 2016; Liu Y等, 2018)、*Ca.*

Heimdallarchaeota和*Ca.* Odinarchaeota(Zaremba-Niedzwiedzka等, 2017)也有使用WL通路的潜力. 最近在这个超门中也发现了具有烷烃代谢潜力的古菌物种, 即*Ca.* Helarchaeota(Seitz等, 2019; Cai等, 2020).

DPANN超门目前在多种环境中均被发现, 该超门古菌通常拥有较小的基因组, 大多数在其生命周期中依赖其他微生物. 近些年发现的新物种包括如*Ca.* Woesearchaeota、*Ca.* Aenigmarchaeota、*Ca.* Pacearchaeota和*Ca.* Diapherotrites(Podar等, 2013; Rinke等, 2013; Probst等, 2014; Castelle等, 2015; Ortiz-Alvarez和Casamayor, 2016; Liu X等, 2018)以及*Ca.* Micrarchaeota和*Ca.* Parvarchaeota(Chen等, 2018), 推测其具有多种代谢潜能. *Ca.* Nanohaloarchaeota(Ghai等, 2011; Narasingarao等, 2012)具有有氧异养或厌氧发酵代谢的潜能. *Ca.* Altiarchaea(Bird等, 2016)可能固定二氧化碳, 代谢小分子有机碳化合物, 如乙酸、甲酸和一氧化碳等.

在这些不依赖于古菌分离与纯培养的研究中, 大部分新发现来自于科学家对不同生态系统中古菌介导地球化学元素循环过程的研究结果. 在此, 我们仅列举数个古菌烷烃代谢方面的研究实例来说明在过去五年中取得的丰硕成果. 甲烷的厌氧代谢过程(包括多碳烷烃代谢)对于全球碳循环的平衡和气候变化具有非常重要的意义(Welte, 2018; Evans等, 2019; Wang等, 2020). 产甲烷过程和甲烷厌氧氧化过程普遍认为是由古菌中广古菌门古菌通过甲烷代谢途径或逆途径进行, 该途径涉及一种关键酶——甲基辅酶M还原酶(MCR). 通过宏基因组分箱的方法对苏拉特盆地(Surat Basin)的煤层气井所获得的数据集进行分析, 发现两个属于深古菌门的MAGs具有编码MCR的基因(Evans等, 2015). 该报道首次表明, 广古菌门外的古菌也可能具有代谢甲烷的能力. 然而, 深古菌的MCR蛋白质序列与典型的甲烷代谢MCR差异较大. 一年后, 另一组研究者用正丁烷而非甲烷独立地完成了从瓜伊马斯盆地所获得沉积物样品的富集培养. 这些研究者发现, 广古菌门中两种新型MAGs含有一种能够氧化正丁烷的MCR(Laso-Pérez等, 2016). 这一发现扩展了对古菌非甲烷多碳烷烃代谢的认识. 同年, 来源于一个新古菌门*Ca.* Verstraetearchaeota的五个MAGs从三联式纤维素厌氧发酵罐被发现, 这些发酵罐内包含来源于瘤胃、湖泊沉积物、厌氧发酵罐、咸水湖的混合材料(Vanwonterghem等, 2016). *Ca.* Verstraetearchaeota的成员拥有*mcr*基因, 推断其可能使用甲基化化合物, 如甲醇和甲胺来产生甲烷. 两项关注甲烷(烷烃)代谢的全球宏基因组调查进一步扩展了*mcr*基因的分布, 其中包含多个潜在的古菌门(即广古菌门、奇古菌门、深古菌门、*Ca.* Verstraetearchaeota、*Ca.* Korarchaeota、*Ca.* Nezhaarchaeota、*Ca.* Hadesarchaeota)(Borrel等, 2019; Wang等, 2019b; Hua等, 2019). 此外, 自发现硫酸盐依赖的甲烷厌氧氧化途径后, 这一过程通常被认为是由硫酸盐还原细菌和甲烷厌氧氧化古菌组成的共生体系所介导. 通过使用宏基因组分箱的方法, 科学家发现广古菌门的Archaeoglobi(Colman等, 2019; Wang等, 2019b)及*Ca.* Korarchaeota(McKay等, 2019; Wang等, 2019b)中的某些成员既有异化硫酸盐还原基因*dsr*, 也有*mcr*基因; 这些报道首次发现将两个代谢过程耦合于同一细胞内的古菌. 简而言之, 有关甲烷(或烷烃)代谢古菌的研究仅仅是所有古菌域最新发现的冰山一角, 在其他各种古菌中, 还有更多未鉴定的代谢过程, 这些代谢也可能有助于更深刻的理解全球生物地球化学元素循环.

4 纯培养古菌与古菌基因组库规模之间的差距

目前普遍认为, 自然界中只有极小部分微生物(估计<0.01~1%)可以在实验室进行纯培养(Staley和Konopka, 1985; Alain和Querellou, 2009), 但该观点仍然存在争议(Martiny, 2019; Steen等, 2019). 通过对比公共基因组数据库和国际上菌株保藏库数据, 可以看到目前在美国国家生物技术信息中心(National Center for Biotechnology Information, NCBI)和整合微生物组数据库(Integrated Microbial Genomes and Microbiomes, IMG)的基因组数据库中, 分别有5989个(属于38纲、25门)和1989个(属于33纲、21门)古菌基因组(2020年11月). 同时, 在德国微生物菌种保藏中心(Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ)和基因组在线数据库(Genomes Online Database, GOLD)中, 分别仅有377株(属于9纲, 2门)和567株(属于10纲, 3门)的古菌模式菌株(2020年11月). 可以看到, 古菌基因组数量显著高于纯培养古菌数量(图2), 此外, 数据库中环境古菌16S rRNA基因数量也比分离

古菌模式菌株16S rRNA基因的数量高几个数量级, 这说明自然界中实际古菌物种数与纯培养古菌物种数之间存在巨大差距.

导致这种差异的原因可能有以下几点: 首先, 许多古菌可能具有互营共生或寄生的生活方式, 单一菌株不能从原位环境微生物群落网络中分离. 其次, 由于一些古菌生活在低能量条件下(即资源有限或难以利用, 如较为贫瘠的深部环境, Hoshino和Inagaki, 2018), 或生长速度极为缓慢(如甲烷厌氧氧化古菌, Nauhaus等, 2007). 这些古菌在自然界中的丰度可能非常低, 而使用常见底物分离培养时, 快速生长微生物的生长速率可能会远超过这些古菌物种, 使得很难排除非目标菌株. 此外, 在自然界中, 很多古菌类群需要严格或较窄的生长条件范围, 而这些生长条件很难在实验室中精确模拟, 因此无法对这些古菌进行培养.

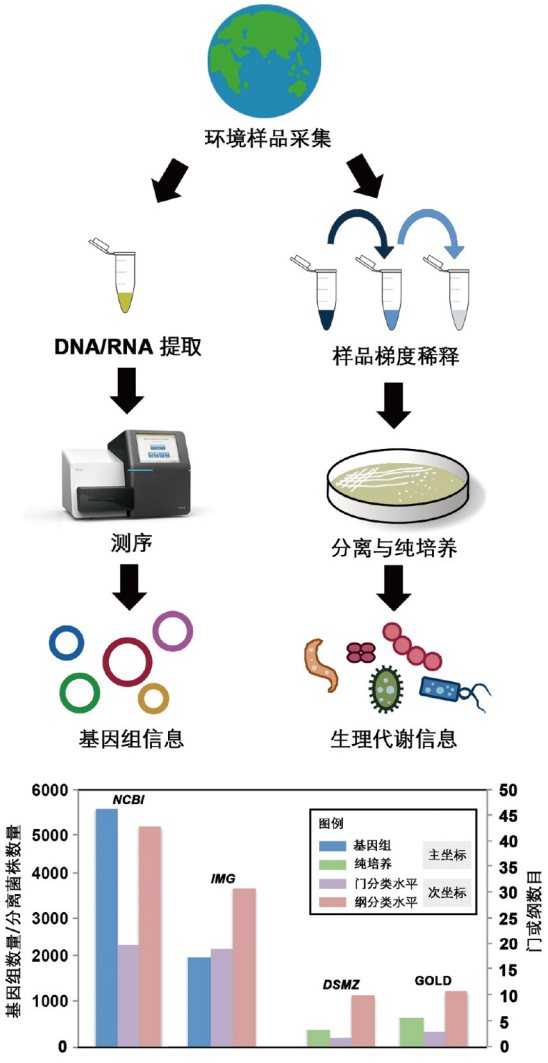

图 2　高通量测序方法研究古菌代谢潜能(左), 以及传统分离与纯培养方法研究古菌生理功能(右)

柱状图显示了不同数据库及菌种保藏中心的古菌基因组和纯培养模式菌株数量

目前研究人员开发出较多新的培养技术来克服古菌菌株分离和纯培养的困难. 新的技术通过与传统技术相结合(Nakamura等, 2009; Sakai等, 2009; Tanaka等, 2014), 发展出微流控(Boitard等, 2015; Kaminski等, 2016), 毛细管、封装技术、光学拉曼钳单细胞操作(Ben-Dov等, 2009; Park和Chiou, 2011), 培养芯片技术(Ingham等, 2007; Hesselman等, 2012)和高通量培养方法, 又称“培养组学”技术(Lagier等, 2012). 虽然这些技术显著增加了纯培养微生物的菌株数量, 但仍然遵循“反复实验和试错”的原则, 或依赖与目标微生物密切相关的其他微生物的培养经验. 由于影响古菌分离和生长的因素非常多, 为了提高这些方法的效率, 本综述提出结合高通量培养与大规模古菌基因组信息作为指导, 来对古菌进行有目标的分离和纯培养工作.

5　基于古菌基因组信息的分离与纯培养策略

随着越来越多古菌基因组及其潜在代谢特征的报道, 这些信息可以用来指导古菌分离和纯培养(Gutleben等, 2018). 一旦这些古菌菌株被成功分离和纯培养, 就可以尝试进行遗传操作系统的构建. 该系统通过构建插入和敲除基因的突变体, 为研究未知或具有重要功能的蛋白质打下基础, 并能够验证生物信息学的预测, 如最近构建的古菌*Pyrococcus yayanosii*中的抗毒素系统(Li Z等, 2018). 随着纯培养古菌的增加, 可以更详细的对其代谢进行研究, 也可以在各种培养条件下对古菌菌株的生理特征进行研究. 例如, 通过对纯培养广古菌门产甲烷古菌的研究, 已经详细揭示了产甲烷代谢通路中关键酶的催化特征(Borrel等, 2016; Scheller等, 2017; Thauer, 2019; Wang等, 2020). 此外, 纯培养古菌高值次级代谢产物的产量也可被优化, 并用于生物技术应用, 例如来自极端嗜热微生物*Pyrococcus furiosus*中获得的Pfu DNA聚合酶等已经完全商业化(Lundberg等, 1991; Ibrahim和Ma, 2017). 本综述提出了基于大量环境基因组信息指导古菌分离和纯

培养的新思路, 即: (1) 基因组功能预测, (2) 代谢互作网络构建, (3) 基因组水平代谢建模, (4) 机器学习预测培养方法, 期望能够在高通量测序所积累数据的指导下, 获得更多古菌模式菌株(图3).

5.1　基因组预测代谢通路有助于分离和纯培养体系设计

基于古菌基因组的信息, 通过京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes, KEGG)(Kanehisa等, 2015)或基于子系统技术的快速注释程序(Rapid Annotations using Subsystems Technology, RAST)(Overbeek等, 2013)等方法来预测和注释基因, 并绘制整体代谢重构图, 进而了解古菌代谢潜能以及相关功能基因的存在和缺失等基本信息. 例如, 如果可以确定目标古菌具有进行碳固定、硝酸盐还原、硫氧化或芳香族化合物降解等代谢的潜力, 研究者可以针对这些预测出的代谢途径设计特定的培养基. 通过基因组获取的信息也可以了解某些化合物不能在目标古菌细胞内合成, 如特定氨基酸和维生素, 需要额外添加到培养基中. 此外, 通过宏转录组及宏蛋白质组的信息, 也有助于研究者结合目标古菌在当前环境物理化学因素下的代谢响应, 来选择其合适的生长条件. 虽然目前通过使用基因组与宏转录组和宏蛋白质组获得的信息来分离古菌的报道较少, 但随着公共数据库中越来越多古菌基因组和其他组学数据的积累, 可预见这种方法在未来将被越来越多地进行尝试.

其中一个实例是来自于一种广泛分布的嗜热嗜酸古菌, 它属于深海热液喷口广古菌门的DHVE2类群(Reysenbach等, 2006). 几十年来, 科学家试图对该古菌进行分离和纯培养的努力一直没有获得成功. 研究发现DHVE2类群与嗜酸菌*Thermoplasma*、*Picrophilus*和*Ferroplasmad*的16S rRNA基因序列非常相似, 这些古菌倾向酸性的生存环境, 具有代谢有机化合物和硫的能力. 基于这些信息, 研究者设计了一种特殊的, pH为4.5, 并含有硫元素、酵母提取物和胰蛋白胨等组分的培养基, 成功完成了富集培养, 在经过几次连续稀释后, 最终分离并纯培养了该古菌类群, 并获得两个DHVE2菌株, 命名为*Aciduliprofundum boonei* T449和T469. 该成果是来自深海喷口DHVE2类群中最先分离的严格嗜热嗜酸古菌(Reysenbach等, 2006).

纳古菌门是基因组指导古菌菌株分离和纯培养的另一个实例. 这类古菌拥有较小的细胞尺寸(100~300nm), 并在多种极端环境中被发现, 如热液喷口、咸水湖和热泉等(Huber等, 2002). 科学家通过采用单细胞分选技术, 对从热泉样本中分离出的微生物进行了高通量测序, 科学家发现了两个纳古菌门的物种和一个广古菌门中Sulfolobales目的未培养物种(Casanueva等, 2008). 该纳古菌门微生物拥有一个极小的基因组, 并且缺乏生物合成的基本途径, 而参与糖酵解和糖异生的基因仍然存在(Podar等, 2013). 这一发现表明, 纳古菌门可能依赖于一个共生微生物, 但仍能够使用糖类作为能源或碳源(Podar等, 2013; Wurch等, 2016). 通过这些信息, 研究者设计出一种含有蔗糖的培养基, 显著提高并稳定了富集培养中的纳古菌门古菌的数量. 研究人员通过光钳将Sulfolobales的单个细胞和一个纳古菌门的细胞转移到培养基中, 从而成功实现纳古菌门(*Ca. Nanopusillus acidilobi*)及其宿主*Acidilobus* sp.的分离及纯培养.

在基因组信息的指导下, 还能够成功获得古菌富集培养物. 例如, 深古菌门古菌是地球上丰度最高的微生物之一, 并且广泛分布于各种环境中, 包括海洋和淡水厌氧沉积物、土壤、生物反应器和动物肠道相关的环境, 深部生物圈等(Kubo等, 2012; Lloyd等, 2013; He等, 2016; Xiang等, 2017). 研究表明, 深古菌门古菌有潜力降解各种复杂有机质, 如蛋白质和芳香化合物, 并水解细胞外多糖类物质, 包括几丁质和木质素等(He等, 2016; Zhang等, 2016; Lazar等, 2017; Zhou等, 2018; Feng等, 2019). 通过使用基因组预测出的底物进行富集培养实验, 如将纤维素、油酸、干酪

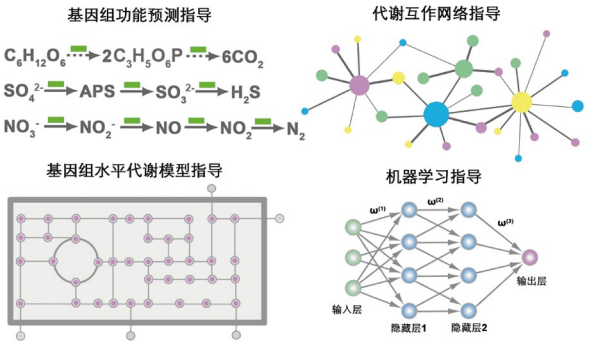

图 3　基因组信息指导古菌分离与纯培养的四种策略

四种策略即基因组功能预测指导、代谢互作网络指导、基因组水平代谢模型指导和机器学习指导

素、苯酚和木质素添加到培养基中，经过长时间的培养，研究人员在木质素添加的河口沉积物样品中发现一个深古菌门亚群的显著富集(Yu等, 2018), 进一步研究说明该古菌可以利用木质素作为能源，同时以二氧化碳作为碳源进行生长.

对分别来自深海热液喷口和热泉的*Aciduliprofundum boonei* T449、T469和*Ca. Nanopusillus acidilobi* N7A的成功分离和纯培养，以及使用木质素对深古菌门亚群的富集，都表明基因组指导的方法在分离培养未知但功能重要的古菌方面具有重要作用.

5.2 微生物间相互作用网络指导古菌分离与纯培养

在自然界中，不同微生物通常聚集在一起组成群落，并且微生物之间具有很强的相互作用，如可能争夺有限的资源，或以协同互利的方式生存(Faust和Raes, 2012). 此外, 在自然环境中, 微生物还可以通过群体感应形成网络. 群体感应是一种对细胞密度变化作出反应的机制，可能是通过交换如有机酸，维生素，甚至电子等代谢物来实现(Ng和Bassler, 2009; Zelezniak等, 2015; Pande和Kost, 2017; Zengler和Zaramela, 2018; Charlesworth等, 2020). 因此, 了解微生物群体间提供的信号分子或代谢物可能有助于分离目标物种(Camilli和Bassler, 2006; Haruta等, 2009; Faust和Raes, 2012; Zelezniak等, 2015).

其中一个经典的相互作用实例来自于产氢细菌和氢型产甲烷古菌之间的相互作用，这种相互作用通常被称为互营(养). 微生物之间的氢气转移对于厌氧细菌和产甲烷古菌都至关重要，对于厌氧产氢细菌，随着氢气浓度的增加，对有机物的发酵过程非常不利，除非产生的氢气被氢型产甲烷古菌利用. 因此，在该情况下，产甲烷古菌能够维持一个厌氧群落，通过这种“共培养法”能够分离出目前未培养的产甲烷古菌，如Rice Cluster I产甲烷古菌类群(Sakai等, 2007, 2008). Sakai等(2009)首先报道了对氢气具有高度亲和力的产甲烷古菌的分离和纯培养，采用的方法是将丙酸发酵产氢细菌用作“诱饵”微生物. 通过类似方法，阿斯加德古菌超门的一个模式菌株，*Ca.* Prometheoarchaeum syntrophicum MK-D1, 和一种产甲烷古菌*Methanogenium*的共培养物在经过长期实验后被成功分离和纯培养. *Ca.* Prometheoarchaeum syntrophicum MK-D1可以为产甲烷古菌*Methanogenium*产生甲酸和氢气, 同时利用其产生的氨基酸和维生素B12作为底物进行生长. 这种古菌互营模式也为真核生物的起源提供了重要的证据，一方面，菌株MK-D1与真核生物在进化树分析中关系紧密，另一方面，由菌株MK-D1形成的细胞结构能够裹挟细菌，暗示真核细胞中线粒体祖先形成的一种潜在机制(Imachi等, 2020).

除了这种可靠而传统的分离方法外，我们还总结了基于古菌代谢相互作用网络指导的三种潜在方法. 首先，直接通过基因组预测的营养缺陷或信号分子通路预测中获得的信息，推测可能形成的相互作用关系，并针对这样的关系具体设计分离和纯培养方案(Vartoukian等, 2010; Sipkema等, 2011). 第二, 微生物之间共生网络的构建可以为目标古菌和其他微生物之间的共生关系提供关键信息. 如果目标古菌与某些可培养微生物高度相关，则可以通过其培养液的无细胞上清液或来自相关可培养微生物的匀浆来强烈刺激目标古菌的生长. 但是目前仍没有关于基于该方法分离出古菌菌株的报道，而一些细菌菌株通过使用其他细菌的上清液已经能够被成功分离(Tanaka等, 2004; Bhuiyan等, 2016; Xian等, 2020). 第三, 目标古菌与相关微生物之间的负相关关系在古菌分离中也具有十分重要的意义(Yim等, 2007; Voolaid等, 2012). 多组学数据不仅可以预测与目标古菌呈负相关的微生物，还可以提供有关特定抗生素信息来抑制与目标古菌共存的快速生长微生物(Rettedal等, 2014).

5.3 基因组水平代谢建模指导的古菌分离与纯培养

基因组水平代谢建模(Genome-scale metabolic models, GEM)可通过基因组数据预测微生物的代谢能力，并且可以提供单个细胞内或微生物群落中的代谢生化反应网络(Bordbar等, 2014; Monk等, 2014; O’Brien等, 2015; Kim等, 2017; Muller等, 2018). 这种方法通过整合多组学数据来建立微生物基因型和表型之间的关系(Feist等, 2006; Richards等, 2016; Dufault-Thompson等, 2017; Li F等, 2018). 这种整合的数据被认为是预测微生物对不同环境条件响应的有效方法. GEM建模过程已经有较多且全面的综述(Oberhardt等, 2009; Bordbar等, 2014; O’Brien等, 2015; Kim等, 2017), 一般来说，首先需要基于精确的基因组注释进行初步

代谢重构; 接下来, 使用这些数据生成一个基础模型, 该模型用于通过现有算法来预测被分析基因组的表型; 随后, 可以通过基于约束条件的方法, 考虑从包括热力学、通量、转录调节和化学计量学等多个方面将这些数据引入模型, 从而准确地预测微生物代谢通量(Reed, 2012; Imam等, 2015; Kim等, 2017). 目前, 几种成熟的工具已被用于直接从基因组信息来获得代谢重建模型(Angione等, 2016; Faria等, 2018; Karlsen等, 2018). 基于单纯基因组预测和GEM方法的区别在于, 基因组信息应用于古菌的分离和纯培养要更简单直接, 而GEM需要数据库中更多的代谢和生化结果来构建一个准确的模型. 虽然目前基于GEM指导的古菌分离和纯培养研究仍然缺乏, 但提高微生物可培养性的潜在研究已有报道. Carr和Borenstein(2012)发表了一个名为"NetSeed"的建模工具, 该模型可以推测微生物在其生存环境中需要的化合物. 根据NetSeed的数据, 最小化环境工具箱(Minimal Environment Tool, MENTO)可以用于预测某些细菌或古菌所需的基础营养方案. MENTO是首个基于GEM指导的培养基设计实用工具(Zarecki等, 2014). 目前, GEM不仅可以针对一种目标微生物, 还能够用于复杂群落的代谢重构, 如菌膜(Zhang, 2017)和人类肠道生态系统(Magnúsdóttir等, 2017). 随着多组学数据的快速增加, 该方面的类似研究将会逐渐普及(Levy和Borenstein, 2013; O'Brien等, 2015; Shapiro等, 2018).

5.4 使用机器学习通过基因组信息预测培养方案

机器学习的定义可追溯到20世纪60年代, 当时Samuel(1959)将其描述为"一个使计算机能够在没有明确编程的情况下进行学习的研究领域". 随后, Mechell以更形象化的方式将机器学习一词定义为"如果用性能测试(P)来评估计算机程序在某类任务(T)上的性能, 一个测试通过使用经验(E)在T任务上获得了性能改善, 那么认为关于T和P, 该程序对E进行了学习"(Michell, 1997). 一般来说, 数据可以分为训练集和测试集. 前者用于训练机器学习模型(使用不同的算法), 而后者用于测试每个模型对于特定问题的处理情况, 例如分类、预测或功能选择. 虽然机器学习在生命科学中并不是一个新概念, 但随着学习材料的数据量日益增加, 如大量的古菌基因组数据, 以及计算机计算能力和学习性能的显著提高, 机器学习在生物研究中的应用可能在不远的将来蓬勃发展(Angermueller等, 2016; Guo等, 2017; Cuperlovic-Culf, 2018). 各种机器学习算法, 包括自组织映射、支持向量机和贝叶斯(Bayesian)网络, 已得到广泛的应用(Schmidhuber, 2015; Angermueller等, 2016; Li等, 2018; Cuperlovic-Culf, 2018). 例如, 深度学习方法(机器学习的分支领域)已成功地用于识别DNA甲基化和组蛋白标记, 以及用于转录调节区域的预测等(Gibbs等, 2010; Montgomery等, 2010; Waszak等, 2015). 此外, 随着多组学数据积累, 机器学习被广泛用于优化代谢网络模型、分析生物反应器的最佳条件以及化学计量动力学模型的优化等(Libbrecht和Noble, 2015; Wu等, 2016; Lin和Lane, 2017; Costello和Martin, 2018).

在本研究中, 我们建议利用机器学习来设计未知古菌菌株的分离和纯培养条件, 通过现有的纯培养古菌基因组信息及其培养基作为训练数据集, 以实现研究者仅提供未培养古菌基因组信息, 即能够得到针对该古菌的预测优化培养基组分. 目前已有一个名为"已知培养基数据库"(Known Media Database, KOMODO)的程序, 该程序包含43300种培养基类型, 以及418000个微生物和培养基组合(Oberhardt等, 2015). 研究者可以使用KOMODO根据新微生物16S rRNA基因序列提供与其最接近微生物的培养基列表. 但是, 在大多数情况下, 微生物16S rRNA基因的相似性和生理特性仍具有较大差距, 同一微生物属中的两个菌株也可能具有截然不同的生理特性, 但是不同门的两个物种也可能具有相似的代谢特征. 因此, 通过机器学习模型的建立, 结合与功能相关的基因组信息、培养基数据以及相关实验结果, 同时将不同因素考虑在内, 如一些古菌物种可能进入可存活不可培养的状态(VBNC), 以及一些复苏因子(如过氧化氢酶, 丙酮酸钠和群体感应因子)可能也对古菌的培养有促进作用(Mu等, 2021; Zhang等, 2021). 在未来, 研究者也许能够同时从基因组和实验数据中收集信息, 以构建训练集, 这些训练集随后被用于提供培养基的可能组分及其浓度, 增大分离和纯培养目标古菌的可能性.

6 展望

古菌分离和纯培养对于理解生命演化及其对地球化学元素循环的影响至关重要. 过去10年以来, 科学家

重点关注了未培养古菌环境生物学方面的研究, 在通过宏基因组学发现新古菌这一方面也已经取得了较大进展, 但如何将预测的古菌物种实现纯培养仍然是一项重大挑战. 为了解决这一问题, 首先, 我们认为传统的古菌分离和纯培养方法, 以及最近发展的新技术, 如“培养组学”, 仍然是强有力的工具, 通过结合仔细和精心修改培养基或培养条件, 通过调整不同的环境因子, 如压力、氧化还原度, 甚至培养时间, 逐步将一个复杂群落缩小为一个简单的群落, 最终实现古菌菌株分离和纯培养; 第二, 通过使用大规模基因组数据, 包括多组学和相互作用网络分析, 将扩展我们对许多新的未培养古菌代谢潜力的理解, 这将为古菌分离和纯培养提供有效指导; 第三, 通过GEM模型的构建, 以及机器学习方法也可能大大加快新古菌菌株的分离和纯培养; 最后, 我们强调耐心和毅力是研究者成功分离和纯培养古菌的先决条件, 因为许多未培养古菌生长速度尤其缓慢. 此外, 政府及研究机构对该领域的资金和人员投入也将显著促进该方向的发展. 简言之, 通过采取以上措施, 古菌分离和纯培养这一基础研究的进展将极大帮助科学家了解生命的起源和演化, 并准确描述生物地球化学过程如何影响地球上的元素循环及可能引起的气候变化.

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