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rRNA-targeted 寡核苷酸探针识别活性污泥微生物群落结构与功能研究进展

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摘要:活性污泥中微生物群落内部关系非常复杂,及时对活性污泥中优势菌群和群落内部关系进行监测是污水处理中采取正确措施的关键。历史研究表明传统培养方法经常导致活性污泥优势菌群检测的失败,而 rRNA-targeted 寡核苷酸探针作为一种快速原位监测活性污泥微生物群落结构和功能的新工具被引入,使我们对参与污水净化的微生物群落结构和优势菌群能有较全面的了解。就该方法在识别除磷污泥、脱氮污泥、污泥泡沫和膨胀污泥中微生物群落结构和功能的典型应用进行综述,分析了该方法存在的优点和缺点,并对目前已建立且应用于活性污泥微生物检测的 rRNA-targeted 寡核苷酸探针进行了详细总结。

关键词:rRNA;寡核苷酸探针;杂交;微生物生态;活性污泥

Application of rRNA-targeted oligonucleotide probes in the analysis of microbial communities in activated sludge

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Abstract: Wastewater treatment by activated sludge is one of the most important biotechnological processes and has been used for more than 80 years. However, the information on correlations between the microbial community structure and function of activated sludge is less provided. Due to intricate interactions within the microbial community, it is important to detect the prominent population and describe microbial community structure in the wastewater treatment process. Conventional cultivation methods often result

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in pronounced population shifts in the community structure and lead to a failure to detect dominant bacterial community memmbers. Recently, rRNA-targeted oligonucleotide probes were introduced as a new powerful tool in situ monitoring microbial ecology in activated sludge and have made revolutionary progresses. Based on it, microbial ecologists can for the first time determine the true composition of microbial communities. This review introduces the identification of it in structure and function of microbial communities in phosphate-removing activated sludge, nitrifying activated sludge, bulking sludge and foaming activated sludge, summarizes most of probes which have been developed and used, analyzes the advantages and disvantages of it.

Key words: rRNA; oligonucleotide probe; hybridization; microbial communities; activated sludge

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用活性污泥法处理污水是当今世界上最主要的污水处理工艺之一,并已有 80 年多年的历史。在工艺工程方面已经投入了大量工作,但是关于活性污泥中起主要作用的微生物的群落结构与功能的关系的知识仍非常有限;这也是微生物生态学家一直非常感兴趣的问题。用传统培养的方法已从活性污泥中分离和鉴定了大量细菌并用此方法对其微生物共生体的多样性和动力学进行分析,但此方法存在许多缺点:①非常耗时,需额外进行生理生化特性的鉴定,在实验室纯培养条件下测定单个种的生理生化特性可能使表现型产生偏差,因基因表达受环境条件和细胞生长模型的的影响[1];②直接显微计数得到的细胞总数与活皿计数法或最大或然数目法(MPN)得到的细胞总数间存在很大差异(后两者分别是前者的 1%～10%),因为环境中大多数微生物是不可培养的;③由此方法得出的结果会引起微生物群落结构的组成在很大程度上发生偏移,从而导致检测优势细菌群落成员的失败[2]。因此试图用直接的方法分析活性污泥中细菌群落结构的特性,如已成功地运用了产醌特性、多胺模型和免疫荧光等方法;但这些方法对纯培养有很强的依赖性。近年来,rRNA-targeted 寡核苷酸探针作为原位鉴定细菌的一种新的工具被引入,与以上方法相比较,它能更快更准确地描述活性污泥中微生物的群落结构[3]。由于活性污泥中微生物群落内部的关系非常复杂,所以对污水处理厂的工艺控制非常难;而且污水处理操作条件的变化会导致微生物群落内部种群的变化并由此而引起污泥质量问题如污泥沉淀特性不好、污泥致密等。因此发展诊断、监测和控制活性污泥问题的探针在污水处理实践中非常有意义。

1　rRNA-targeted 寡核苷酸探针

1.1　rRNA-targeted 寡核苷酸探针原位监测活性污泥微生物群落结构和功能的原理

以微生物体内的 rRNA 为靶序列,通过人工化学手段或 PCR 扩增等方法来合成带荧光或放射性标记 DNA 寡核苷酸探针,用此探针与活性污泥中微生物体内的 rRNA 原位杂交,用荧光落差显微镜、放射自显影、流式细胞仪或激光扫描共聚焦显微镜等手段对杂交信号进行检测和扫描并用相关统计软件进行分析其与活性污泥类型和运转效能间的关系,或建立数学模型用于预测活性污泥的运转状况。

1.2　rRNA-targeted 寡核苷酸探针的优点

(1)大多数细胞内存在大量的 rRNA,在有代谢活性的细胞内 rRNA 是以 10^4 到 10^5 的拷贝数自然扩增的[4],因此用寡核苷酸探针检测活性污泥可以原位提供单个细胞的鉴定和活性。

(2)rRNA 核苷酸序列既包括了高度保守的序列又含有易变的区域;因此 rRNA-targeted 寡核苷酸探针可在微生物系统发育的不同水平上自由调整如从亚种到整个微生物界[5]。

(3)此方法建立在 rRNA 分子序列的比较分析基础上,目前已建立了用于 rRNA 序列比较分析的庞大数据如 RDP(Ribosomal Database Project)等和用于分析和设计探针的软件包[6,7]如 ARB(Latin,"arbor" = tree)等。其中 ARB 数据库包括 22 000 个线性核糖体小亚基 RNA 和 500 个核糖体大亚基 RNA 序列,用 ARB 软件包可以以线性的形式设计 rRNA-targeted 寡核苷酸探针[8],而且已建立了寡核苷酸探针数据库并可网上浏览查阅[9]。

1.3　杂交中应注意的问题

(1)杂交信号的强度与细菌细胞的生长率密切相关[10],因为细胞内 rRNA 含量依赖于细胞所处的生理状态。如果某些菌核糖体含量太低就可能不被检测到,但该方法能检测到的细胞总数(70%～80%)远远高于培养的方法(1%～10%)。如果因细胞内核糖体含量低而不能被检测到,很可能是因为这些细菌原位特性不活跃和不重要[12]。因此杂交 rRNA 的丰度可以代表特定微生物种群的原位相对生理活性,对研究微生物生态具有重要意义。

(2)固定剂的选择很重要,因 G^+ 和 G^- 菌的细胞壁的结构不同,一般乙醇较适合固定 G^+ 菌而多聚甲醛适合固定 G^- 菌,因此样品需用两种固定剂同时固定[13]。

(3)通过加入竞争性探针可鉴别单个碱基的错配,提高杂交的灵敏度[14]。

1.4 rRNA-targeted 寡核苷酸探针的缺点

(1)rRNA 的多样性只是被部分地描述[15]。

(2)寡核苷酸探针分子较短,所带标记物较少,灵敏度相对较低,但合成容易,成本低,易于商品化。

2 rRNA-targeted 寡核苷酸探针在识别活性污泥微生物群落结构和功能中的应用

2.1 rRNA-targeted 寡核苷酸探针在除磷污泥中的应用

在生物除磷工艺中,起生物除磷作用的是污泥中的积磷菌,包括分类和系统发育上多种多样的菌,且大多数是不可培养的[16]。最早用培养的方法认为积磷菌主要成员是不动杆菌(*Acinetobacter* spp.)(γ 亚纲变形菌),然而纯培养中的不动杆菌很难协调碳和磷的转化,这与生物除磷工艺中的生化机制不同[11,17],不动杆菌 16srRNA-targeted 寡核苷酸探针的应用表明其不是积磷菌中的优势菌[18,19]。rRNA-targeted 寡核苷酸探针荧光原位杂交(FISH)与培养方法结合对除磷活性污泥中微生物群落结构进行调查,杂交结果表明优势菌群为 β 亚纲变形菌和 G+C 含量高的 G^+,而不动杆菌不到总菌数的 10%;但用培养的方法得到的菌落杂交表明 30%以上的为不动杆菌,因营养丰富的平板有利于 γ 亚纲的生长,而不利于 β 亚纲和 G+C 含量高的 G^+[20];其它相关研究均表明不动杆菌所占比例相对较低,而 β-2 亚纲的红环菌(*Rhodocyclus* spp.)在除磷污泥中占很大比例,可能红环菌在生物除磷中起重要作用[21];3 个积磷菌寡核苷酸探针的成功设计应用及直接、间接和定量的数据均表明 β-2 亚纲的红环菌和丙酸杆菌(*Propionibacter* spp.)是除磷污泥中除磷菌的重要成员[19,22];一种已知的积磷菌-积磷小月菌(*Microlunatus phosphovorus*)在群落中占的比例很小,但它很可能在生物除磷污泥中起作用[23]。探针和聚磷酸盐 DAPI(4',6-diamidino-2-phenylindole)双染色法的应用更提高了积磷菌检测的可靠性[24,25]。

2.2 rRNA-targeted 寡核苷酸探针在脱氮污泥中的应用

在微生物脱氮系统中硝化作用的提高是影响脱氮效率的一个关键。实验室硝化反应器中荧光光谱法检测氨氧化菌探针和亚硝酸盐氧化菌探针杂交信号发现此两种菌的比例为 2.3∶1[26]。由于硝化细菌世代时间长,菌落易形成聚集体而难以计数,因此很难用培养的方法进行研究。在 16s rRNA 序列比较分析的基础上,已经建立了关于一些自氧氨氧化菌探针并成功地用于污水处理厂中这些菌的原位监测[27]。Mobarry 等设计了氨氧化菌和亚硝酸盐氧化菌系统发育限定种的一系列 5 个 16s rRNA-targeted 寡核苷酸探针用于分析硝化污泥中硝化细菌的数量和空间关系,硝化污泥的定量狭线印迹杂交和全细胞杂交结果均表明在污泥絮凝体中存在着亚硝化单胞菌(*Nitrosomonas* spp.)和硝化杆菌(*Nitrobacter* spp.)且两者经常相互接触[28];但是在其它的研究中却未在活性污泥中检测到亚硝化单胞菌和硝化杆菌,而是发现氨氧化菌主要是活动亚硝化球菌菌或亚硝化螺菌(*Nitrospira moscoviensis*)、亚硝酸盐氧化菌主要是硝化螺菌类似菌(*Nitrospira*)[29,30];且亚硝酸盐去除能力高低的两种活性污泥中微生物群落分子特点不同[31]。反硝化生丝微菌属(*Hyphomicrobium*)DNA/DNA 杂交群 HG27 是同时和间歇硝化和反硝化相结合污水处理厂活性污泥中优势反硝化生丝微菌属种群的重要成员之一[32];Gliesche 等设计了 HG27 特异性寡核苷酸探针 Hvu-1[33];Christian 等用此探针在硝化和反硝化相结合的污水处理厂对此种群进行了 15 个月的调查,发现 HG27 种群的数量大约占活性污泥兼性厌氧菌总数的 30%,环境条件的变化与此种群数量的季节性变化无显著的相关性[34]。Real-timePCR 的发展和应用并与原位杂交相结合使活性污泥中特定微生物种群的定量研究成为可能,并对硝化污泥中关键微生物-硝化螺菌进行了定量研究[35];以寡核苷酸探针为主的分

子生物学方法和数学方法的结合建立了关于反映硝化污泥结构和功能的数学模型,能将工艺运行数据直接转化为反映微生物群落结构和功能的参数[36]。

2.3 rRNA-targeted 寡核苷酸探针在检测引起污泥膨胀细菌中的应用

在活性污泥法污水处理厂运行问题中,90%是由污泥膨胀引起的。导致活性污泥膨胀的细菌从形态上可以分为丝状菌和非丝状菌两类。通过形态和简单染色的方法,Eikelboom 描述了活性污泥中 26 种丝状菌[37],但这种鉴定方法有它的缺陷如①微生物形态和染色反应在很大范围内与环境条件有关,②丝状硫发菌属(*Thiothrix* spp.)、Eikelboom021N 和毛霉状亮发菌(*Leucothrix* spp.)几乎不能通过形态来区分,且数量低形态特征不明显的丝状菌容易被丢失。rRNA-targeted 寡核苷酸探针可以对可能引起污泥膨胀的细菌进行快速的原位监测,保证及时有效地采取措施减少污水处理厂的经济损失。活性污泥中的 G^- 丝状菌主要有束缚杆菌属(*Haliscomenobacter* spp.)、球衣菌属(*Sphaerotilus* spp.)、纤发菌属(*Leptothrix* spp.)、硫发菌属、亮发菌属和 Eikelboom021N,它们使二沉池中活性污泥絮凝体沉降特性不好导致污泥膨胀;Wagner 等建立了上述各菌群的 16rRNA-targeted 寡核苷酸探针并和激光扫描共聚焦显微镜结合用于活性污泥的检测,即使在固定絮凝体的中心也能检测到丝状菌且不受自发荧光等问题的干扰[38]。寡核苷酸原位杂交可以提高丝状菌尤其是菌丝较短者的检出和鉴定,可以将几乎不能通过形态来区分开丝状硫发菌属、Eikelboom021N 和毛霉状亮发菌区分开,并在污泥实际监测中得以应用[39,40]。对引起污泥膨胀的丝状菌的系统发育和生理特性的研究是制定控制膨胀策略所必需的,膨胀污泥中常见丝状菌 Eikelboom0041 及相连菌的系统发育和生理特性的 FISH 和 MAR(放射性自显影)原位研究表明它们系统发育的多样性和代谢底物的不同[41]。

2.4 rRNA-targeted 寡核苷酸探针在污泥泡沫中的应用

污泥泡沫是污水处理厂存在的一个普遍而严重的问题,它阻碍固液分离降低出水质量,风吹起含病原菌的泡沫还可能危害人们的健康。污泥泡沫的存在和发生与含分枝菌酸的放线菌密切相关,尤其是放线菌中的污泥戈登式菌(*Gordona. amarae*)通过形态和生理的方法被证明是泡沫中的优势菌。因大多数丝状放线菌形态相似,依赖于传统的记数和鉴定方法很难为泡沫问题找到合理的解决方法。Francis 等建立分枝杆菌群(*Mycobacterium*)、戈登式菌属和污泥戈登式菌嵌套式特异性探针对活性污泥系统的丝状泡沫进行特性研究,发现几种污泥戈登式菌(仅占现存戈登式菌的很小一部分[42],而且在不同污水处理厂污泥戈登式菌 rRNA 的数量和类型都有所变化[43]。戈登式菌的世代时间长,活性污泥中戈登式菌单个细胞 rRNA 含量是高度变化的,因此可将寡核苷酸探针和抗体染色的方法相结合对单个细胞进行鉴定和活性估计,来提高其检测的特异性[44];用上述方法已建立了定量估计污泥泡沫中戈登式菌生物量的方法[45];Oerther 等用该方法对泡沫形成前、中、后期的丝状微生物进行了定量研究,发现泡沫形成过程中戈登式菌的生物量和活性明显增加,但占总 rRNA 库的比例很低,虽然在数量上占优势,但在总生物量中的代谢活性有限,因此在丝状泡沫中戈登式菌的作用可能与丝状微生物的存在比与细胞的代谢活性的相关性要强[46]。

3　活性污泥微生物检测中应用的 rRNA-targeted 寡核苷酸探针

现将从 rRNA-targeted 寡核苷酸探针用于活性污泥(主要是以上 4 种污泥)微生物监测到目前为止所有建立并应用的探针总结如下 (见表 1),探针的设计、应用和发展表明该方法研究的进步。

4　展望

活性污泥微生物生态学是当前生态学领域的研究热点之一,rRNA-targeted 寡核苷酸探针原位杂交是研究活性污泥这一复杂微生物共生体的理想工具之一。随着 rRNA 序列数据和寡核苷酸探针数据库的不断庞大,所设计探针的特异性越来越强,从而可对活性污泥中的微生物进行更细致、更深入、更准确地原位定位。该方法与其它方法结合如显微放射自显影、PCR-DGGE、流式细胞仪检测、激光扫描共聚焦显微镜观察和基因克隆等将使微生物生态学家能够估计工艺参数变化对活性污泥微生物群落结构的影响。特定种群的原位计数能预测故障(如丝状菌引起的膨胀)并及时地提供重新正确运行的机会。

如果欲对活性污泥微生物群落的组成和多样性进行长期监测可先用 rRNA-targeted 寡核苷酸探针原位杂交和 PCR-DGGE 电泳进行微生物数目和 DNA 遗传多样性的整体研究;然后在用网式探针杂交,即使

表 1 rRNA-targeted 寡核苷酸探针名称、序列、靶位点和特异性

Table 1 rRNA-targeted oligonucleotide probe name, sequence, target site, specificity

探针名称 Probe	序列 Sequence(5'-3')	靶位 rRNA target site[a]	特异性 Specificity	资料来源 References
EUK516	ACCAGACTTGCCCTCC	16S,502-516	Eucarya	[14]
EUB338	GCTGCCTCCCGTAGGAGT	16S,338-355	Bacterial	[47]
EUB338-Ⅰ	GCAGCCACCCGTAGGTGT	16S,338-355	Bacterial	[48]
EUB338-Ⅱ	GCTGCCACCCGTAGGTGT	16S,338-355	Bacterial	[48]
ALF1b	CGTTCG(C/T)TCTGAGCCAG	16S,19-23	αsubclass of *proteobacteria*, several members ofδsubclass of *proteobacteria*, most spirochetes	[12]
BET42a	GCCTTCCACTTCGTTT	23S,1027-1043	Bsubclass of *proteobacteria*	[12]
GAM42a	GCCTTCCACATCGTTT	23S,1027-1043	γsubclass of *proteobacteria*	[12]
CF319a	TGGTCCGTGTCTCAGTAC	16S,319-336	*Cytophaga-flavobacterium* cluster of cytophaga-flavobacterium-bacteroides phylum	[49]
HGC69a	TATAGTTACCACCGCCGT	23s,1901-1918	G^+ with high G+C DNA	[50]
BONE23a	GAATTCCATCCCCCTCT	16S,663-679	β1 subclass of *proteobacteria*	[51]
ACA23a	ATCCTCTCCCATACTCTA	16S,652-669	Acinetobacter spp.	[20]
ACA652b	ATCCTCTCCAATACTCTA	16S,652-669	Some unknown *Acinetobacter*-like species	[51]
ARC94	TGCGCCACTTAGCTGACA	16S,94-111	*Arcobacter* spp.	[52]
ARC1430	TTAGCATCCCCGCTTCGA	16S,1430-1447	*Arcobacter* spp.	[52]
RHC175	TGCTCACAGAATATGCGG	16S,175-192	*Rhodocyclus* cluster	[53]
RHW456	CGGGTATTAACCGAAGCA	16S,456-473	*Rhodocyclus*-like clone R1	[53]
RHW991	GTTCTCTTTCGAGCACTC	16S,991-1008	*Rhodocyclus*-like clone R1	[53]
RHX456	AGGGTATTAACCCAAGC	16S,456-472	*Rhodocyclus*-like clone R6	[53]
RHX991	GCTCTCTTGCGAGCACTC	16S,991-1008	*Rhodocyclus*-like clone R6	[53]
Rc988	AGGATTCCTGACATGTCAAG GG	16S,988-1009	Rhodocyclus group	[54]
PAO462	CCGTCATCTACWCAGGGTAT TAAC	16S,462-485	PAO cluster	[54]
PAO651	CCCTCTGCCAAACTCCAG	16S,651-668	PAO cluster	[54]
PAO846	GTTAGCTACGGCACTAAAAGG	16S,846-866	PAO cluster	[54]
NIT3	CCTGTGCTCCATGCTCCG	16S,1035-1048	*Nitrobacter* spp.	[55]
NSO190	CGATCCCCTGCTTTTCTCC	16S,190-208	Ammonia-oxidizing in the βsubclass of *proteobacteria*	[28]
NSO1225	CGCCATTGTATTACGTGTGA	16S,1225-1244	Ammonia-oxidizing in the βsubclass of *proteobacteria*	[28]
NSV443	CCGTGACCGTTTCGTTCCG	16S,444-462	*Nitrosospira* spp.	[28]
NSM156	TATTAGCACATCTTTCGAT	16S,156-174	*Nitrosomonas* spp.	[28]
NSR826	GTAACCCGCCGACACTTA	16S,826-843	Freshwater *Nitrospira* spp.	[30]
NSR1156	CCCGTTCTCCTGGGCAGT	16S,1156-1173	Freshwater *Nitrospira* spp.	[30]
NMV	TCCTCAGAGACTACGCGG	16S,174-191	*Nitrosococcus mobilis*	[29]
S-*-Ntspa-1026-a-A-18	AGCACGCTGGTATTGCTA	16S,1026-1043	*Nitrospira moscoviensis*	[29]
S-G-Hypho-1241-a-19	GCTGC(G/C)CATTGTCA CCGCC	16S,1241-1260	*Hyphomicrobium* genus	[56]
Hvu1034	GCACCTGTCCCACTGCCT	16S,1034-1051	*Hyphomicrobium. vulgare*	[57]
HHY	GCCTACCTCAACCTGATT	16S,655-672	*Haliscomenobacter hydrossis*	[38]
LDI	CTCTGCCGCACTCCAGCT	16S,649-666	*Leptothrix discophora*	[38]
LMU	CCCCTCTCCCAAACTCTA	16S,652-669	*Leucothrix mucor*	[38]
SNA	CATCCCCCTCTACCGTAC	16S,656-673	*Sphaerotilus natans*	[38]
TNI	CTCCTCTCCCACATTCTA	16S,652-669	*Thiothrix nivea*	[38]
21N	TCCCTCTCCCAAATTCTA	16S,652-669	Eikebloom 21N	[38]
S-*-Myb-0736-a-A-22	CAGCGTCAGTTACTACCC AGAG	16S,736-757	*Mycibacterium* complex	[42]
S-G-Gor-0596-a-A-22	TGCAGAATTTCACAGACG ACGC	16S,596-617	Gordona	[42]
S-S-G. am-0912-a-A-18	CACCCACCCCCATGCAGG	16S,192-209	*Gordona. amarae*	[42]
S-*-G. am1-0439-a-A-19	TCGCGCTTCGTCCCTGGTG	16S,439-457	*Gordona. amarae* group 1 strains	[43]
S-*-G. am2-0439-a-A-19	CGAAGCTTCGTCCCTGGCG	16S, 439-457	*Gordona. amarae* group 2 strains	[43]

[a] Escherichia coli numbering

用相分类单元特异性探针进行从大到小过筛式的方法对活性污泥进行研究:(1)使用细菌域或古菌域特异性探针;(2)用变形细菌纲各亚纲(α、β、γ)及其它谱系的探针;(3)用属的特异性探针杂交;(4)用相应属合适的培养基和培养条件进行分离培养和生理生化鉴定(纯培养是对细菌的生理和功能进行详细分析的先决条件,在探针检测的基础上选择合适的培养基和培养条件,可以提供工艺运行的微生物和生化方面更进一步的信息);把微生物的动态变化与原生动物的动态变化及水质资料相联系,分析微生物、原生动物的动态变化与工厂运行条件和性能的关系,发展诊断、监测和控制活性污泥问题的探针,希望得到可靠和预测性的相关指示种群来监测和预示工厂的运行状况。或者是用 rRNA-targeted 寡核苷酸探针原位监测某种活性污泥中起重要作用的某种优势菌群的动态变化,研究它们与污水处理厂运行参数和水质参数的相互联系建立数学模型可用于监控和预测该种污泥的运行状况。总之,活性污泥微生物群落结构和功能与污水处理厂运行状况之间关系的研究有许多尚待解决的问题,rRNA-targeted 寡核苷酸探针在该领域的引入引起了该领域革新性的变化。

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