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大气颗粒物中微生物分析方法及分布特征的研究进展

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摘要: 大气颗粒物(particulate matter, PM)可能携带病原微生物,会对人体健康产生影响,因此,对大气颗粒物中微生物的采集和分析已成为当今的研究热点。本文对大气颗粒物中微生物的采集方法和分析方法(培养法、菌群多样性组成谱测序和宏基因组学技术)的研究进展进行简要概括,着重介绍不同条件下(区域、时间、粒径、天气条件和气象因素)大气颗粒物中微生物的分布状况差异,并对引起相关差异的原因进行了探讨,旨在为深入认识大气颗粒物中微生物的分布特征提供参考。

关键词: 大气颗粒物;微生物;分析方法;分布特征
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Advances in Analytical Methods and Distribution Characteristics of Microorganisms in Particulate Matter

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Abstract: Accumulating evidence suggests that particulate matter could carry pathogenic microorganisms which had adverse effects on human health, and thus current studies have been focusing on the collection and analysis of pathogenic microorganisms in particulate matter. The collection and analysis methods (culture method, microbial diversity compositional spectrum sequencing, and metagenomics technology) of microorganisms in particulate matter were summarized briefly in this study. Furthermore, the different distribution characteristics of microorganisms in particulate matter in different conditions (spatial, time, particle size, weather conditions, and meteorological factors) were reviewed. The causes of the differences among these distribution characteristics were then analyzed to provide a reference for further understanding of the distribution characteristics of microorganisms in particulate matter.

Keywords: particulate matter; microorganisms; analytical methods; distribution characteristics

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近年来,我国很多地区灰霾天气频发,引起社会公众的普遍关注。大气颗粒物(particulate matter, PM)是大气中组分复杂的污染物质,影响了全球气候变化和大气过程[1-2]。PM 不仅可以通过其有毒有害的化学组分危害人体健康,还可通过其携带的微生物诱发疾病[3]。已有临床研究证实 PM 中含有病原微生物和抗生素抗性基因,严重危害易感人群的健康,因此,采集 PM 并对其中的微生物进行鉴定和分析显得尤为重要[4-5]。目前较为常用的采集方法有过滤采样、碰撞采样和液体冲击式采样等[6];研究表明,PM 中微生物的分析方法可分为培养法和非培养法[7-8]。培养法作为标准方法使用较为广泛,但难以分析分离培养困难的菌群[9];而非培养法克服了这一缺点,近年来发展迅速,尤其是宏基因组学技术,不仅可分析种及以下水平的菌群,还能检测新基因[10]。

PM 中微生物的分布特征受很多因素的影响,如区域、时间、粒径大小、天气条件和气象因素等[11-15]。其中季节变化对微生物的影响较大,且大多数研究认为微生物浓度在夏季和秋季较高,冬季和春季较低[12,16-18];微生物群落结构在区域分布的差异主要归因于地区间气候条件和地形地势等自然因素以及功能区划等人为活动[13,19-20];微生物在不同天气条件下的分布也有所差异,比如沙尘天气下微生物浓度高于非沙尘天气[15,21-22]。本文概述了目前常用的 PM 中微生物的采集方法和分析方法,重点阐述了 PM 中微生物的分布特征及其影响因素,旨在为深入理解 PM 中微生物的组成及相关政策法规的制定提供理论依据。

1 大气颗粒物中微生物的采集方法(Methods for collecting microorganisms in particulate matter)

PM 中微生物的采集方法主要有过滤采样、碰撞采样和液体冲击式采样等[2,6-7,23],其中碰撞采样可估算微生物的数量,但有些微生物不能被粘附到琼脂表面使得其对微生物的采集效率降低[6,23];而液体冲击式采样对微生物的采集效率高,但采集到的微生物容易失活且对病毒的采集效率低[24-25];与碰撞采样和液体冲击式采样相比,虽然过滤采样过程中微生物孢子易被破坏,但因其具有采集效率高、价格经济等优点,在 PM 中微生物的采集方面使用最为广泛[6,26]。目前,采集微生物常用的滤膜材料有石英纤维[27-29]、玻璃纤维[4,8,30]和聚碳酸酯等[7,31],其中石英纤维膜因其颗粒物捕集效率高、熔点高且耐酸性强等优势被广泛应用于 PM 中微生物的采集[31-33]。

2 大气颗粒物中微生物的分析方法(Methods for the analysis of microorganisms in particulate matter)

目前,PM 中微生物的分析方法可分为培养法和非培养法,其中非培养法包括不基于聚合酶链式反应(polymerase chain reaction, PCR)技术的非培养法(如磷脂脂肪酸法和染色-荧光显微镜测定法等)和基于 PCR 技术的非培养法(如菌群多样性组成谱测序和宏基因组学技术等)[7-8]。培养法作为标准方法被广泛用于 PM 中微生物的研究,但其很难反映微生物群落的真实多样性[34]。而基于 PCR 技术的非培养法可用于分析很多难以培养的微生物,近年来发展迅速,推动了 PM 中微生物的相关研究[35]。与培养法相比,以菌群多样性组成谱测序和宏基因组学为代表的基于 PCR 技术的非培养法可提高对微生物群落结构的认识,为全面研究 PM 中微生物的分布特征提供基础[7-8,36]。总的来说,培养法可检测部分可培养微生物的种类和数量,菌群多样性组成谱测序可分析微生物的群落结构,而宏基因组学不仅可分析和鉴定难以培养的微生物的种类和数量,还可用于研究微生物的功能[37-38]。

2.1 培养法

培养法在国外起源较早,在国内起步虽晚但发展迅速,目前广泛应用于环境中微生物的研究[39-42]。平板培养法作为常用的培养法,广泛用于 PM 中微生物的研究[16,19,28,30],如 Gou 等[30]使用平板培养法研究发现乌鲁木齐市 PM_{10} 中可培养细菌和真菌的浓度与 PM_{10} 的浓度和温度有显著的正相关关系。由于菌属对培养基的配制具有选择性,使得 99% 以上的微生物均为未被纯培养或难以纯培养,但其因具有简单和经济的特点目前仍被广泛使用[42]。

2.2 菌群多样性组成谱测序

2.2.1 16S rRNA 基因测序

16S rRNA 基因测序是通过提取环境样本的 DNA,选择合适的通用引物对特定高可变区进行扩增,反映微生物的物种组成及群落多样性,常用于细菌和古菌群落的研究[43]。研究表明,16S rRNA 测序不仅能为我们研究微生物群落结构提供大量信息,还可有效鉴定分离培养困难及生长缓慢的菌类[44]。但 16S rRNA 测序也存在一定缺陷,如其存在多拷贝的异质性和基因的水平转移等问题,这些都直接影响了微生物群落结构和多样性的分析[45]。

2.2.2 ITS 扩增子/18S rRNA 基因测序

ITS 扩增子/18S rRNA 基因测序是通过提取环

境样本的 DNA,选择合适的通用引物扩增特定区域,检测目标区域的序列变异和丰度,目前被广泛用于真菌群落的研究[46]。两者在适用范围方面有所差异:ITS 扩增子测序中度保守,但进化速率快,能够提供较丰富的变异位点,更适用于属内或种内差异较明显的菌群间的研究[47-48];18S rRNA 测序测定区域比较保守,更适用于科内或种间系统发育关系的相关研究[49-50]。

2.3　宏基因组学技术

宏基因组学技术是从环境样本中直接提取基因组遗传物质,分析其微生物群落的遗传信息[51]。近年来,宏基因组学发展迅速,其中较为常用的技术有 Illumina、基因芯片和末端限制性片段长度多态性分析等(表 1)。宏基因组学技术不仅可从属种级别上

表 1　常用微生物宏基因组学技术比较

Table 1　Comparison of several widely-used metagenomics techniques in microorganisms

分类 Classification	代表性技术 Representative techniques	优点 Advantages	缺点 Disadvantages	参考文献 References
DNA 分子指纹图谱 DNA-fingerprinting	限制片段长度多态性分析 Restriction fragment length polymorphism analysis	(1)可快速鉴别菌群所属的物种区系;(2)可间接鉴定微生物的功能 (1) Rapid identification of the microorganisms flora; (2) Indirect identification of the microbial function	(1)无法检测低丰度微生物的种类;(2)多样性评估受到序列组成的影响,对特定微生物的检测限高 (1) Could not detect the low-abundant microorganisms; (2) The assessments of diversity are affected by the sequence composition. High detection limits for specific microorganisms	[56-57]
微阵列技术 Microarray	基因芯片 Gene chip	(1)可分析微生物的功能;(2)灵敏性高,通量高 (1) Able to analyze functional gene; (2) High sensitivity and throughput	属于封闭系统,仅可用于检测已知的基因信息 Only the known genetic information detectable	[58]
随机测序 Random sequencing	全基因组鸟枪法测序 Whole genome shotgun sequencing	(1)速度快,操作简单;(2)可反映微生物的组成、群落结构和代谢潜力 (1) Simple and rapid; (2) Able to demonstrate the composition, community structure and potential metabolic capacity of microorganisms	(1)序列的组装和拼接复杂,分析过程易出现错误;(2)分析时缺乏比对的数据库 (1) Error-prone complex steps for assembly and splicing of sequences; (2) Lack of comparable database	[59,6]
高通量测序 High-throughput sequencing	Illumina	(1)速度快,通量高;(2)可分析微生物的多样性和丰度 (1) Rapid and high-throughput; (2) Able to analyze the microbial diversity and abundance	(1)物种和基因丰度可能被高估;(2)易受污染物干扰 (1) Overestimating the species and genetic abundance of microorganisms; (2) Susceptible to pollutants	[37,60-61]
稳定性同位素标记 Stable isotope labeling	荧光原位杂交技术 Fluorescence in situ hybridization	(1)可确定可培养和不可培养的微生物的生理状态;(2)可进行特异微生物跟踪检测 (1) Able to determine the physiological state of cultivable and non-cultivable microorganisms; (2) Capable of tracking detection for specific microorganisms	(1)灵敏度低;(2)难以分析染色体总的核型 (1) Low sensitivity; (2) Difficult to analyze the total karyotype of chromosomes	[62-63]
	流式细胞仪 Flow cytometry	(1)可分析的样本量大;(2)检测速度快;(3)结果准确 (1) Able to analyze large samples; (2) Rapid; (3) Accurate	(1)仪器价格昂贵;(2)样本前处理过程复杂 (1) Expensive instruments; (2) Complicated preparation processes of samples	[64]

发现难以分离培养的菌群,还可用于研究微生物群落的功能[52-55]。此外,宏基因组学技术也可用于检测 PM 中致病菌的种类,如 Cao 等[27]应用宏基因组学技术分析了北京市 PM 中的微生物,发现 PM 中存在可导致肺炎的典型致病菌(如肺炎支原体(*Streptococcus pneumoniae*))。

3 大气颗粒物中微生物的分布特征(Distribution of microorganisms in particulate matter)

PM 中微生物的浓度和群落结构随区域、时间、粒径、天气条件和气象因素等的变化呈现出一定的差异,可能是受到地形、气候、污染物类型和浓度及功能区类型等因素影响[16,19-20]。

3.1 大气颗粒物中微生物的时间分布特征

PM 中微生物浓度和群落结构是不断变化的,几天之内甚至同一天的不同时间段也有所差异[65-66]。Fierer 等[65]分析了美国科罗拉多州博尔德市连续 8 d 的 PM,发现细菌与真菌的比例在采样期间变化了 2 个数量级。陈铭夏等[66]研究了南京市 PM 中微生物,发现微生物浓度在同一采样天内有所差异,其中细菌浓度在 5:00 和 20:00 均有一个最大值,真菌浓度则在 9:00 和 24:00 左右达到最大值。研究发现季节变化对微生物的影响较大,不同季节微生物浓度和群落结构均有所差异[17-18,67-68]。有研究表明微生物在夏季和秋季浓度较高,冬季和春季浓度较低,其中夏季微生物浓度高的原因可能是微生物在高温条件下易通过蒸发作用从土壤和水体迁移到大气,使 PM 中微生物浓度增加;而冬季微生物浓度较低则可能是微生物在低温环境下易形成孢子等休眠体,从而抑制了微生物的生长和繁殖[12,16-18]。不同季节,PM 中微生物丰度也有所不同,如 Gandolfi 等[67]研究表明,意大利米兰市和威尼斯市的 PM 中伯克氏菌目(Burkholderiales)和放线菌目(Actinomycetales)丰度在寒冷季节较高,而 Chloroplasts 则在温暖季节相对丰富;Bowers 等[68]研究发现美国科罗拉多州 PM 中真菌在春季和夏季较为丰富,而细菌则在春季和秋季较为丰富。总之,微生物的浓度和种类在不同时间存在一定差异,但总体来说其在夏季和秋季浓度较高,冬季和春季浓度较低。

3.2 大气颗粒物中微生物的空间分布特征

PM 中微生物的浓度和群落结构在不同地区存在一定差异[69-71]。例如中国香港以蓝细菌属(*Corynebacterium*)为主,美国中西部地区以拟杆菌属(*Bacterium*)为主,意大利则以放线菌属(*Actinomyces*)为主[70-71]。此外,PM 中微生物的优势菌属在同一地区不同功能区的分布也有所差异[16,19],如王琳[19]研究了青岛市不同功能区 PM 中微生物的分布特征,发现冬季市区街道细菌的优势菌属为不动杆菌属(*Acinetobacter*)和弓形杆菌属(*Arcobacter*),其中不动杆菌属为常见致病菌;而人工湿地 PM 中微生物则以马赛菌属(*Massilia*)和鞘脂单胞菌属(*Sphingomonas*)为主,其中鞘脂单胞菌属为与植物有关的致病菌。不同地区 PM 中微生物浓度有所差异[72-76]:例如美国辛辛那提市(184 ~ 16 979 spores·m^{-3})和中国花莲市(1.33×10 ~ 8.55×10^4 spores·m^{-3})真菌孢子浓度远远高于澳大利亚(1.7×10^2 ~ 3.2×10^3 spores·m^{-3})[74-76]。此外,不同粒径的 PM 中微生物的分布也有区域上的差异,如新加坡地区真菌浓度在粒径范围为 1.1 ~ 3.3 μm 的 PM 中最高,而中国敦煌、奥地利和地中海地区的真菌则主要存在于粒径大于 2.1 μm 的粗颗粒物中[77-78]。PM 中微生物的浓度和群落结构在不同地区有所差异,且其优势菌属在同一地区不同类型的功能区内也有所不同。

3.3 不同粒径下大气颗粒物中微生物的分布特征

不同粒径的 PM 可沉积于人体的不同部位,因此有必要研究不同粒径下 PM 中微生物的分布特征[79-83]。不同粒径的 PM 中微生物的浓度有所差异,且大多数研究认为微生物主要分布在粒径较大的颗粒物(>2.1 μm)中,而在粒径较小的颗粒物中浓度较低[73,80-81]:如李婉欣等[80]研究发现西安市 PM 中细菌大多分布在粗颗粒物(>2.1 μm)中,且其浓度会随着颗粒物粒径的增大而增加;韩晨等[73]研究表明青岛市 PM 中真菌易吸附在粗颗粒物(>2.1 μm)表面;Alghamdi 等[82]研究也表明沙特阿拉伯吉达市 PM_{10} 中放线菌属(*Actinomycetes*)浓度(8 604 CFU·m^{-3})远高于 $PM_{2.5}$(100 CFU·m^{-3})。此外,不同粒径下 PM 中微生物的优势菌群较为相似,但微生物的丰度有所差异[28,81-83]:如 Xu 等[28]的研究表明济南市不同粒径的 PM($PM_{0.18 \sim 0.32}$、$PM_{0.32 \sim 0.56}$ 和 $PM_{0.56 \sim 1}$)中主导的细菌在不同分类水平上较为相似,但是其丰度存在差异,如醋菌属(*Acetobacter*)和全噬菌科(Holophagaceae)在 $PM_{0.32 \sim 0.56}$ 中丰度最高,分别约为 $PM_{0.18 \sim 0.32}$ 的 5 倍和 6 倍;Yan 等[83]研究发现北京市不同粒径的 PM 中均以枝孢属(*Cladosporium*)和链格孢属(*Alternaria*)占主导,且总的来说真菌丰度和多样性在总悬浮颗粒物(total suspended particulate matter, TSP)中较高,PM_{10} 次之,$PM_{2.5}$ 最低;黄琼[81]

研究也表明,南昌市 PM_{10} 中放线菌属(*Actinomycetes*)丰度(70%)高于 $PM_{2.5}$(20%)。不同粒径下 PM 中微生物的浓度有所差异,总的来说,微生物在粒径较大的颗粒物中浓度较高,不同粒径下微生物的优势菌群的群落结构具有很强的相似性,但其丰度存在差异[28,80-83]。

3.4　不同天气条件下大气颗粒物中微生物的分布特征

PM 中微生物的浓度和优势菌属不仅受时空和颗粒物粒径的影响,其在不同天气条件下也有所差异[21-22,84-87]。研究表明,微生物的浓度受到沙尘天气的影响,如 Li 等[21]研究发现,青岛市有尘天气下 PM 中可培养微生物浓度为无尘天气下浓度的 222.8%,可能由于沙尘或细颗粒物可作为微生物的载体并为其提供营养,导致微生物浓度升高。此外,研究发现沙尘和非沙尘天气条件下 PM 中微生物的优势菌属有所差异,如 Jeon 等[22]研究表明韩国首尔市沙尘天气下 PM 中优势菌属为厚壁菌门(Firmicutes),而非沙尘天气下优势菌属为变形菌门(Proteobacteria)。

灰霾天气下微生物的浓度高于非霾天气,如 Li 等[84]研究发现,西安市灰霾天气下 PM 中微生物浓度(细菌:1 102.4 ~ 1 736.5 CFU·m^{-3};真菌:1 466.2 ~ 1 703.9 CFU·m^{-3})远高于非霾天气(细菌:497.7 ~ 629.0 CFU·m^{-3};真菌:247.6 ~ 398.4 CFU·m^{-3})。但是也有研究与之相反,如 Gao 等[41]研究发现,北京市非霾天气下 PM 中可培养的微生物浓度高于灰霾天气。

此外,PM 中污染物的种类和浓度会影响微生物的分布[29,84-86],Han 等[85]研究发现,北京市 PM 中的主要污染物(PM、NO_2、SO_2、CO 和 O_3 等)在一定浓度下既可提供载体供微生物生长,也能为微生物的繁殖和发育提供营养,但当污染物浓度较高时会对微生物有毒害作用,使微生物浓度下降。Dong 等[87]研究表明,青岛市灰霾天气下 PM 中微生物的浓度与 PM、SO_2、NO_2 和 CO 等的浓度成正相关性,而与 O_3 浓度则有一定的负相关性,可能是 O_3 与空气中的烯烃及其他物质反应后对微生物的毒性作用增强,使其对微生物的毒性效应占主导地位,从而使微生物的浓度下降[76,87-88]。

PM 中微生物的浓度和优势菌属的分布受到不同天气条件的影响,就微生物浓度分布而言:一方面 PM 可以为微生物的生长和繁殖提供营养,使微生物浓度增加;另一方面,当 PM 中污染物质达到一定值时其毒性作用占主导,使得 PM 中微生物浓度下降。

3.5　不同气象因素下大气颗粒物中微生物的分布特征

国内外许多研究发现 PM 中微生物的浓度和群落结构与气象因素有一定相关性[89-91],如西安市 PM 中微生物浓度在降水和非降水期间有所差异,呈现降水期间(251±180 ~ 194±85 CFU·m^{-3})<非降水期间(晴天(341±158 ~ 254±123 CFU·m^{-3})、阴天(382±216 ~ 312±178 CFU·m^{-3})和灰霾(1 311±371 ~ 896±559 CFU·m^{-3}))的趋势,降水期间微生物浓度变化的原因可能是 PM 中微生物容易受到雨水冲刷的作用,使微生物浓度减少[84]。温度和相对湿度对微生物的影响有所不同,温度主要影响微生物的多样性,尤其是真菌孢子的释放,而相对湿度则主要影响微生物的浓度,大多数研究发现,温度升高和湿度增加均会为微生物生长提供有利的生存条件,使微生物浓度增加[90-93]。此外,适宜的太阳辐射强度可能导致微生物孢子的释放,从而增强了微生物的活性,但较强的太阳辐射对微生物有灭活作用[94]。有研究发现风速是 PM 中微生物传播的动力,可以促进土壤和水体中的微生物附着于 PM,也可以使 PM 中的微生物进入土壤和水体,当风速较大时 PM 向土壤和水体中的迁移作用占主导,使 PM 中微生物的浓度减少[14,95]。气象因素对微生物分布的影响十分复杂,在适宜条件下可能会促进微生物的生长和繁殖,但是其在一定条件下对微生物有灭活作用,使微生物浓度减少。

4　展望(Future research prospects)

近年来,我国 PM 污染问题十分严重,已有研究表明,大气颗粒物中的微生物对人体有潜在的健康风险[1,4]。目前我国对于 PM 中微生物的浓度和群落结构的相关研究主要集中在大城市且多为短期监测,而近年来中小城市以及农村地区的 PM 污染问题越来越严重,但是相关研究相对较少[34,96-97],因此,在未来的研究中仍需对不同功能区以及不同气候类型区域进行长期深入研究,并重点关注典型污染地区不同粒径 PM 中微生物的时空分布特征,这对于评价大气环境质量以及潜在的暴露风险具有重要意义;此外,未来还需从分子水平在气象因子和 PM 的化学组分等因素对 PM 中微生物群落结构以及微生物对 SO_2、NO_2 和 O_3 等大气污染的响应机理等方面进行深入探索,尤其是通过宏基因组学技术研究

PM 中参与碳、氮和硫代谢(如固碳、氨氧化、硝化和反硝化以及脱硫等作用)的功能基因。此外,虽然已有部分研究检测出 PM 中存在致病菌,但对其来源、临床机理及其与污染物和气象因素的相互作用机制尚待进一步深入探索。

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