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Review 综 述

微生物气溶胶检测技术的研究进展

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摘 要：生物气溶胶中微生物的种类复杂多样，其采集和鉴定是全面掌握微生物气溶胶生物特性的关键，对防控气溶胶病原体传播十分重要。本文简要综述了微生物气溶胶的生物特性和潜在危害性，介绍了微生物气溶胶的一般采集方法，采集器采集生物气溶胶的基本原理、特点和优缺点。将有助于研究人员根据实验目的在采集低浓度生物气溶胶时选择合适的采样器。本文围绕气溶胶微生物依赖和不依赖于培养的分析鉴定、单细胞水平鉴定等方法综述了气溶胶微生物的鉴定技术，以及鉴定气溶胶中病毒的研究进展。最后针对现有的微生物气溶胶采集和鉴定方法提出展望，以期为发展微生物气溶胶收集方法和促进对生物气溶胶生物特性的全面理解提供理论基础。

关键词：生物气溶胶；采集方法；鉴定方法

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Technologies for microbial aerosol sampling and identification: a review and current perspective

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Abstract: The bioaerosol harbors diverse microorganisms. The sampling of microbial aerosols and the identification of microorganisms in aerosols are the key to understanding the biological characteristics of microbial aerosols, which is essential for preventing bioaerosol-mediated pathogen transmission. This article briefly reviewed the biological characteristics and potential hazards of microbial aerosols and introduced the general methods for microbial aerosol sampling with respect to their performance, operating mechanisms, advantages, and limitations, in an aim to assist the design of best practices for bioaerosol sampling. Three major (culture-dependent, culture-independent, and single-cell) techniques for the identification of bacteria, fungi, and viruses in bioaerosols were expounded. Finally, the future techniques for microbial aerosol collection and microbial identification were prospected. The cross-disciplinary interaction will facilitate the development of novel methods for bioaerosol sampling and detection. With this review, we hope to provide a theoretical basis for the development of sampling methods and comprehensive characterization of bioaerosols.

Keywords: bioaerosol; sampling method; identification method

气溶胶(aerosols)一般指悬浮于气体介质中的固体或液体颗粒物体系，颗粒物尺寸通常为1 nm–100 μm。含有生物物质的气溶胶被称为生物气溶胶(bioaerosols)，常见的生物微粒包括细菌、古菌、真菌孢子、病毒、藻类、虫卵和花粉颗粒等[1–2]，其中含有微生物(细菌、真菌和病毒)的气溶胶也被称为微生物气溶胶。生物气溶胶不仅具备气溶胶的特性还具有其独特的生物特性，对生态系统和人类健康具有重要的影响，是近些年研究的热点之一。微生物气溶胶具有大范围快速传播疾病的潜在危害性而备受关注。有效地收集和准确地分析微生物气溶胶中所含的微生物是研究微生物如何通过气溶胶传播疾病的前提所在，本综述着重介绍微生物气溶胶采集和分析鉴定的研究进展。

1 微生物气溶胶的生物特性及潜在危害性

随着世界范围内，布鲁氏菌感染、流感、重急性呼吸系统综合征(severe acute respiratory syndrome，SARS)、中东呼吸综合征(middle East respiratory syndrome，MERS)和新型冠状病毒(coronavirus disease 2019，COVID-19)等病原微生物的传播，微生物气溶胶的危害被引起广泛的关注。微生物气溶胶的微生物浓度、种类和丰度极大地受到地域、季节、湿度、温度、风向和风速等因素的影响。一般认为微生物气溶胶中微生物的数量在 10^1–10^6 个细胞/m^3[3–5]。由于环境的动态变化、微生物气溶胶的采集和鉴定方法的差异致使不同研究报道的微生物的种类和数量差

异巨大。细菌、真菌或病毒可以以独立个体或聚集体的形式在空气中扩散[6]，真菌通常以孢子的形式悬浮于空气中，细菌一般吸附于颗粒物表面[7]，当生物形成聚集体(例如形成生物膜结构)时可以有力地抵制大气中的紫外辐照和臭氧等外界环境的压力。气溶胶的微环境为微生物个体或聚集体提供一定的保护作用，而气溶胶微环境的营养成分、水分含量、酸碱度、盐离子成分和温度变化也将影响微生物的活力。

气溶胶中的微生物致敏性、感染性和毒性等是人们关注的重点。有研究报道，大气颗粒物中可以检测到 23 种人类病原菌的基因序列，在大气 PM2.5 时病原菌的相对丰度最高[8]，可吸入病原菌的相对丰度随 PM 污染程度而增加[9]。吸入的生物气溶胶沉积在呼吸道表面或肺部，当达到病原菌感染剂量时可能引发诸如过敏性疾病、呼吸道疾病、神经系统疾病、癌症和 COVID-19 等疾病[10–12]。尽管微生物气溶胶的化学成分、老化程度、粒子形状、直径大小、带电性、密度、温度和吸湿性等因素会影响微生物气溶胶的感染力和致病力，但感染力和毒力的决定性因素是其所携带的微生物种类。含有不同菌株的微生物气溶胶在相似条件下，感染剂量可相差若干个数量级。因此，研究与监测微生物气溶胶中微生物的种类、浓度、分布及其变化规律对防控有害微生物气溶胶，促进人类健康具有重要的意义。

2 微生物气溶胶的收集与鉴定

气溶胶中微生物的检测和鉴定是研究其性质和评估其危害性的前提和依据，相关新技术和新方法的研发目前已成为微生物气溶胶研究中的重要研究方向之一，下面将对微生物气溶胶检测所涉及的采样和分析鉴定两个关键步骤的技术方法进展进行综述。

2.1 微生物气溶胶的采样方法

高效、全覆盖地收集微生物气溶胶是准确获取气溶胶中微生物种类、组成和丰度信息的基础前提。微生物气溶胶与非生物气溶胶的采样原理和方法基本一致，即根据气溶胶的尺寸、重量和电荷等物理特性设计采样方法。常用的采样方法有重力沉降、惯性冲击、过滤、静电采集和冷凝采集等[13]。与非生物气溶胶不同的是微生物气溶胶的研究聚焦于微生物，一般从细胞活力、可培养性和遗传物质完整性等方面评估微生物气溶胶的采样方法，根据研究内容和目的选择合适的采样方法[14]。目前，微生物气溶胶研究领域常采用以下取样方法(表 1)。

2.1.1 重力沉降法

重力沉降法是通过将固体培养基(沉降板)放置于空气环境中，借助气溶胶中微生物自身重力沉降的收集方法，是最简单经济的采样方法。但是该采样方法属于被动采集，误差大，收集效率低，易受颗粒质量、沉降速率、温度、气流和微生物是否可培养等因素影响，难以准确反映空间环境中的微生物总量。由于该方法易于收集较大颗粒的气溶胶，所以仍然被广泛用于室内和室外空气质量评估[15]，以及污水处理厂气溶胶暴露风险评估[16]等研究。若将沉降板和其他主动采样器结合，所得数据将能更准确地反映气溶胶中微生物的总量[17]。

2.1.2 惯性撞击法

惯性撞击法是指利用气溶胶微粒的惯性撞击，将其收集到固体基质或液体中。目前基于惯性撞击的取样设备主要包括冲击采样器(impinger)、撞击采样器(impactor)和旋风分离器(cyclone)三类。冲击采样器，例如 AGI-30 和 BioSampler，含有气溶胶的空气流通过喷嘴加速后使微生物粒子撞击收集液界面，通过收集腔内的收集液捕获气溶胶中微生物(图 1A)。冲击采样器的流速一般为 10–20 L/min[18]。冲击采样器

表 1　不同微生物气溶胶采集方法比较

Table 1　The comparison of different microbial aerosol sampling methods

Collection methods	Characteristics	Samplers	Environments, targets, or biomass	References
Sedimentation	Passive collection, easy for large particles Culturable microorganism	Solid medium	Office, laboratory: bacteria 8.9×10^4 CFU/m^3, fungi 5.0×10^4 CFU/m^3	[15]
	Influence by the environment	Solid medium	Hospital wastewater treatment plant: bacteria 100–400 CFU/m^3, fungi<100 CFU/m^3	[16]
Impinger	Liquid medium, high sampling and retention efficiency Low airflow Collection efficiency was affect by bubbles	BioSampler	Office, laboratory, 10^4–10^5 CFU/m^3	[15]
		SKC BioSampler, AGI-30	*Bacillus subtilis*, *B. atrophaeus*, *Penicillium chrysogenum* test strains	[19]
Impactor	Solid medium	Impactor of multi-slit nozzles	Particles (0.02–2 μm)	[21]
	Single stage or cascade impactor, measurement of size-fractionation of particles Low airflow or high airflow	Andersen impactor	Influenza virus: 6.09 $\log_{10}$ copies of RNA/L	[33]
Cyclone	High collection efficiency for large particles High airflow High mechanical stress	Wet cyclone	H1N1, H3N2	[26]
		NIOSH	H1N1, H3N2: 3.84 $\log_{10}$ copies of RNA/L	[33]
Filtration	Collection on the surface of dry filters	Glass fibre, polycarbonate, gelatin	DNA yield	[30]
	Low airflow Portable but low efficiency for viruses	Polytetrafluoroethylene filter	H1N1, H3N2: 3.66 $\log_{10}$ copies of RNA/L	[33]
		Gelatin filters, polytetrafluoroethylene, polycarbonate	Dust Sampler	[34]
		Mixed cellulose ester, polyethersulfone, polyamide, polytetrafluorethylene and polyvinylidene fluoride	Comparison of five membrane filter	[35]
Electrostatic precipitator	Suitable for collecting virus aerosol	HAFES	Virus aerosols (H1N1, H3N2, MS2)	[36]
	Collection the particles sensitive to electricity Low airflow, high collection efficiency	TB Hotspot DetectOR (THOR)	*Mycobacterium tuberculosis*	[37]
		TracB	particles (0.01–10 mm), *Bacillus thuringiensis* var. *kurtsaki*	[38]
	Corona damages RNA or protein	PEBS	*B. atrophaeus*, *P. chrysogenum*	[40]
Condensation	Suitable for small size particles	Condensation growth tube	*B. subtilis*	[42]
	Collection for virus aerosol High efficiency for capturing live cells or viruses	Viable virus aerosol sampler (VIVAS)	MS2, collection efficiency 74%	[44]
		VIVAS	Viable H1N1, H3N2 and influenza B viruses	[45]
		Spot sampler	Bacteriophages and influenza virus	[46]
		VIVAS	SARS-CoV-2, $TCID_{50}$ 6–74 units/L	[47]

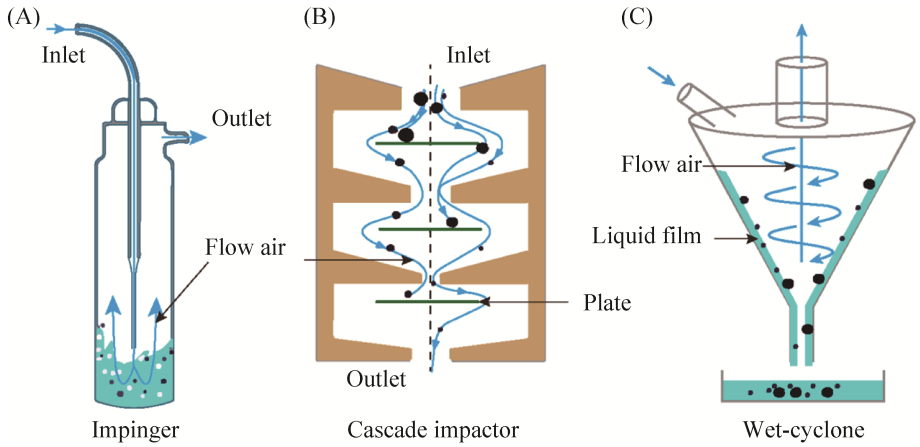

图 1　惯性撞击法采集器原理图

Figure 1　Schematic diagram of inertial impact collector. A: the particles in the incoming airstream were impacted into the liquid collection medium inside the bottle[28]; B: the aerosol particles were classified and collected onto different size stages by successively decreasing nozzle size[28]; C: the particles were collected into an inner-wall liquid film of the wet-cyclone by the particles centrifugal force[29].

的喷嘴结构决定了气溶胶颗粒在收集界面的入射角度和速度；收集腔室的结构也会影响气流的旋转和运动方向；空气流速、采集时间和收集液类型等影响气溶胶的总收集量和总蒸发量，以上因素都将影响气溶胶中微生物的收集效率、保留效率和微生物活性。因此，在采集生物气溶胶样本时需要考虑收集对象的尺寸范围，调整空气流速，优化收集液体积和收集时间来提高收集效率和微生物的生物活力[19]。收集的生物气溶胶样本可以直接用于细胞培养、计数和分子实验等；然而冲击采样器的空气流速较低，收集过程产生的剧烈气泡和液体蒸发将降低收集效率[20]。

撞击采样法将微生物气溶胶引导至喷嘴并提高粒子惯性，气溶胶中粒子撞击于琼脂培养板、滤膜或其他固体基质[13]。撞击采集器可以由多层级串联，构成级联撞击采样器，喷嘴直径和截留的颗粒尺寸随层级的增加而逐渐变小(图 1B)，可以获得气溶胶的粒径分布信息。撞击器的收集效率主要取决于颗粒直径和密度，喷嘴的数量和直径，喷射口的空气速度和收集时间[20]。撞击采样器的空气流速范围一般在 2–170 L/min[18,21]，新型撞击采集器的空气速度甚至可以达到 1 200 L/min[22]。然而高速撞击压力往往会降低微生物活力，撞击板表面的粘合力也会影响微生物的收集效率[23]。

旋风采样器的原理是将带有气溶胶的空气通过螺旋、旋涡等形状的收集室后形成螺旋形旋流。在旋流中，气溶胶颗粒受到与其直径、密度和速度成比例的离心力，该离心力将颗粒带至旋风分离器壁，在分离器壁处颗粒与气流分离并被收集。旋风采样器的入口角度和旋风筒长度影响粒子的收集效率[24–25]。旋风采集器的空气流速可以达到 1 000 L/min[26]，然而高流速往往会损伤微生物细胞，降低微生物纯培养率。湿氏旋风采集器可以提高气溶胶中微生物粒子的捕获效率和生物活性(图 1C)，对直径小于 1 μm 的颗粒物的收集效率略低，对大于 3 μm 的颗粒物的收集效率可达到 100%[27]。本实验室在液体撞击法的基础上设计研发了旋转液腔微生物气溶胶采样器，通过收集液的旋转增加液体的收集面积和颗粒物的离心力，当空气中微生物气溶胶颗粒与旋转液面接触后，水的旋转力会相对柔和并快速地富集微生物气溶胶颗粒。该方法的收集效率与旋风采样器相仿，且在微生物的可培养性上有大幅的提高。

2.1.3 过滤收集法

微生物气溶胶通过由纳米孔结构(例如聚碳酸酯膜)、复杂空隙结构(例如聚氯乙烯膜、凝胶膜)或随机排列结构(例如玻璃纤维膜)构成的滤膜时大颗粒物将被截留于过滤器上(图 2A)[30]。空气通过膜的气流速度通常在 1–133 L/min[18]。研究过程中须根据微生物尺寸大小和实验目的选择不同孔径或材质的滤膜。滤器上的微生物可以转移至固体或液体培养基，也可以用于显微观测、计数、鉴定、DNA 提取和高通量测序分析等[31–32]。过滤器的滤膜材质有聚碳酸酯膜、凝胶、聚氯乙烯膜、聚四氟乙烯膜和玻璃纤维膜等[30,33]。滤膜的材质和结构影响微生物收集效率，例如，聚碳酸酯膜收集细菌的效率明显优于聚四氟乙烯膜和凝胶膜[34]；聚醚砜膜回收细菌、真菌 DNA 的比例高于混合纤维素膜、聚酰胺纤维膜、聚四氟乙烯膜和聚偏二氟乙烯膜[35]。过滤收集可用于依赖培养或不依赖培养的研究，具有成本低和便携等优点，其主要缺陷是膜空隙小易被堵塞，对病毒的回收效率较低，干燥的膜表面可能降低微生物活性。

2.1.4 静电采集法

静电采集(electrostatic precipitator，EP)已经越来越多地应用于病毒和细菌气溶胶收集[36–38]。微生物气溶胶通过静电采集器时首先在静电场入口处被带上电荷，在电场的作用下颗粒与空气流分离并沉积在带电板上(图 2B)。收集效率随空气流速的增加而降低，在低流速下静电采集器可以高效地收集不同尺寸的颗粒物，且机械应力损伤小。在相同温度、湿度和收集时间下静电采集法富集微生物菌落数和总 DNA 的效率均明显高于过滤和液体撞击法[39]。与 BioSampler 相比，Han 等[40]开发的生物气溶胶静电捕集装置(personal electrostatic bioaerosol sampler，PEBS)，捕获活的微生物总量基本一致，而收集气溶胶化的 *Bacillus atrophaeus* 和 *Penicillium chrysogenum* 真菌孢子的总量更高。在静电捕获微生物时还需要防止微生物 RNA 或蛋白质由电晕电离空气产生活性氧造成的损伤等[41]。静电采集法更适合捕获气溶胶中带电敏感或较小尺寸的微生物，其收集效率与电压、流速、除尘器尺寸形状和颗粒带电量等因素密切相关。因其功耗低，可为低功率监测技术提供可行的解决方法。

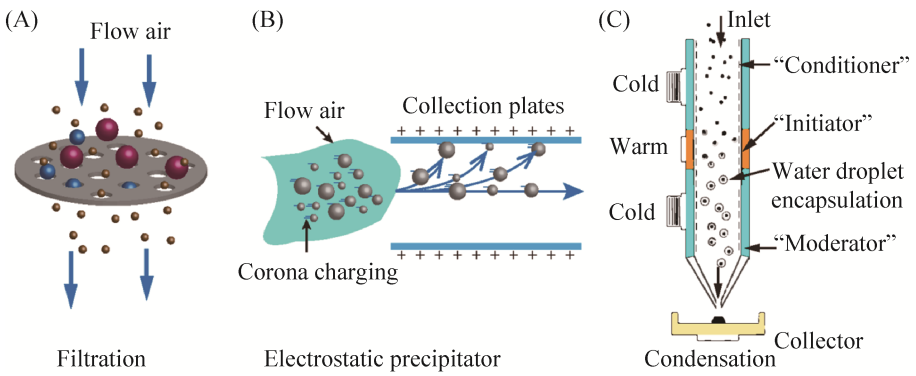

图 2　过滤、静电和冷凝采集原理图

Figure 2　Schematic diagrams of filtration, electrostatic precipitator and condensation sampler. A: particles larger than the diameter of nanopore were collected onto filter media; B: the charged particles were drew to collection plates by electrostatic attraction; C: the particles were introduced into a cold "conditioner", warm "initiator", and cool "moderator" region[42].

2.1.5 冷凝采集法

冷凝采集(condensation sampler)使微生物气溶胶颗粒经加湿器潮湿化和升温后注入冷却器，含有过饱和蒸汽的微生物气溶胶颗粒充当凝结核凝结于冷却器表面(图 2C)。冷凝采集器的升温区与冷凝区温差越大捕获的微生物总量越多，但是高温可能会造成微生物细胞的损伤。与冲击采集器和撞击采集器相比，冷凝采集器的流速相对较低，在收集较小尺寸的微生物方面更具优势，可以实现微生物气溶胶遗传物质的高效率、高保真回收[42]。因此，冷凝采集法多应用于病毒气溶胶的收集。冷凝采集器收集 MS2 病毒的效率比 BioSampler 高 10 倍以上[43]，收集感染性 H1N1 效率比 BioSampler 高 13 倍[44]。VIVAS 冷凝采集器可以在病人 2 m 以外的距离高效地捕获 H1N1 和 H3N2 等呼吸道病毒，而 BioSampler 分离病毒的频率远低于冷凝采集器[45]。冷凝采集器(spot sampler)回收感染性 MS2、PhiX174、Phi6 等噬菌体和气溶胶化 H1N1 流感病毒的效率高于 0.8 μm 聚碳酸酯滤膜[46]。同样地，VIVAS 冷凝采集器收集 COVID-19 患者病房空气气溶胶样品，经 Vero E6 细胞培养后细胞发生病变，结果证明了气溶胶中分离的 SARS-CoV-2 基因组序列与 COVID-19 病人的基因组序列一致[47]。

2.2 微生物气溶胶分析鉴定

微生物气溶胶广泛存在于大气中，成分十分复杂，同时受制于采样技术，从气溶胶中完整无损地分离微生物存在一定的难度，加之气溶胶中存在不可培养微生物，且存在提取单个微生物细胞核酸困难等难题，分析和鉴定一直是微生物气溶胶研究的重点发展方向，因此亟需发展新的分析鉴定技术，以便更加深入地了解微生物气溶胶。本文主要从依赖和不依赖培养以及单细胞分析鉴定方面阐述气溶胶微生物的分析鉴定研究(表 2)。

2.2.1 依赖于培养的分析鉴定

微生物的总量、种类和具有活性的微生物是生物气溶胶研究的重要内容之一。气溶胶样品通过转移至合适的固体或液体中培养和分离纯化，通过统计菌落形成单位(colony forming units，CFU)即可粗略评估气溶胶中可培养微生物的生物量，确定气溶胶活细菌和真菌丰度信息等。基于培养的分析鉴定一般包括纯培养、分离、宏观描述、显微描述和生理生化测试等一系列实验操作。例如，基于培养鉴定原理的全自动细菌鉴定仪(BD Phoenix-100)监测到病人探视活动会增高重症监护室空气中病原微生物总量和耐药菌比例[48]。虽然该方法被用于检测医疗机构[49]、动物诊所[50]等环境中微生物气溶胶，但其耗时长、实验操作复杂。目前气溶胶中微生物的鉴定较多采用分子手段，即提取单菌落 DNA，通过细菌 16S rRNA 基因或真菌 18S rRNA 基因等序列相似性初步判定细菌种类，揭示与污染环境相关的细菌多样性差异[51]，评估医院空气质量，监测空气中人类病原菌等[52]。MALDI-TOF MS 也可以分析空气中微生物种类信息，例如 MALDI-TOF 分析鉴定医院病房和校园宿舍空气中微生物的复杂性[53]。Jäckel 等从生长的菌落中提取总 DNA 并扩增 16S rRNA 基因，最后将混合 PCR 产物用于后续的克隆和测序[54]，该方法可以克服目前基于培养鉴定技术的一些缺陷。然而由于培养条件的限制，并非气溶胶中所有的微生物都可以被纯培养，地球上的微生物目前大概只有 1% 在实验室中培养成功。另外，气溶胶的收集过程可能造成细胞损伤，死亡或处于活的但无法培养(viable but nonculturable，VBNC)状态的微生物也无法纯培养。据估计，灰尘中细菌总量不超过总颗粒物的 10%[55]，可培养的微生物数量不超过总颗粒物的 1%[56]，基于培养的方法获得的细菌总量比非培养方法预测的细菌总量低

表 2　可培养和免培养检测方法比较

Table 2　The comparison between culture and culture-free identification methods

Identification methods	Characteristics	Methods	Collection parameters	Environments	Target or biomass	References
Dependent on culture method	Culturable microorganisms Time consuming, low efficiency	BD Phoenix-100	Andersen Cascade Impactor, 28.3 L/min, 10 min	Intensive care unit	168 CFU/m^3	[48]
		Catalase test	Polytetrafluoroethylen filter, 28.3 L/min, 5 min	Hospital	43–243 CFU/m^3	[49]
		VITEK 2	Ventilation system, total 200 L	Animal clinic	Bacteria 1 000± 800 CFU/m^3, fungi 324±245 CFU/m^3	[50]
		MALDI-TOF MS	Sedimentation	Hospital PM2.5 and PM10	*Staphylococcus*, *Micrococcus*, *Corynebacterium* et al.	[53]
		Sanger sequencing	Whatman filter, 30 min	Hospital	459–1 392 CFU/m^3	[52]
		Sanger sequencing	DLPI+, Polycarbonate filter, HiVo sampler	Livestock facility	18–38 species	[58]
Independent culture method	Base on microbial nucleic acid High throughput, accurate assessment of biomass Unable to distinguish the physiological state of cells	DAPI, LIVE/DEAD BacLight	Polycarbonate filter, 28.3 L/min, 30 min	Air quality index (AQI)	10^4–10^5 CFU/m^3	[3]
		DAPI	Polycarbonate filter, 5 L/min, 24 h	Outdoor PM2.5–PM10	10^4–10^5 CFU/m^3	[32]
		FISH	Polycarbonate filter, 9 L/min, 23 h	Amazon rain forest	1–5×10^5 CFU/m^3	[60]
		Illumina	Quartz filter, 100 L/min, 24 h	Wastewater, submicron aerosols	7 036 virus species, 32 antibiotic-resistance genes	[65]
		Illumina	HighBioTrap, 1 000 L/min, 20 min	13 different land types	10^3–10^5 CFU/m^3	[64]
		Illumina	Polycarbonate filter, 15–60 min	Mountainous and urban areas	10^3–10^5 CFU/m^3	[62]
		Illumina	Quartz filter, 7 d	Global	10^4–10^6 CFU/m^3	[5]
		Illumina	Andersen sampler, 28.3 L/min, 5 min	Indoor	2.3×10^3–8.5×10^3 CFU/m^3	[63]
		Illumina and Oxford Nanopore	HVAC filter	Indoor	Diversity at genus and species level	[66]
Single cell identification	Dependent on the substance within cells In the experimental stage	Surface-enhanced Raman spectroscopy, SERS	Optofluidic SERS	5 test strains	Limit of detection is 10^2 CFU/mL	[76]
		Klarite SERS	Andersen sampler 28.3 L/min, 10 min	Restaurant	*E. coli*	[78]

100–1 000 倍[57–58]。尽管依赖培养的分析鉴定方法具有一定的局限性，但是培养是深入研究微生物生理生化特性、侵染力、毒力、抗逆、耐药性和基因变异等研究的基础。利用多学科和多技术的交叉实现难培养微生物的纯培养对气溶胶微生物的研究意义极其重大，针对气溶胶微生物纯培养技术的开发也将是气溶胶微生物技术发展的重点方向之一。

2.2.2 不依赖于培养的分析鉴定

基于微生物核酸的研究能够规避微生物的纯培养。气溶胶中活的微生物，不可培养的或具有完整形态的死细胞也均可以通过荧光方法定量分析。通过 DAPI (4′,6-diamidino-2-phenylindole)、LIVE/DEAD® BacLight 等荧光染料对细胞膜或细胞核染色和计数可以估测气溶胶中微生物的总量和死活细胞比例等[3]。利用荧光原位杂交技术(FISH)可以原位靶向细胞内 DNA 或 RNA，快速获得细菌和真菌的高阶分类元信息，进而评估室内或自然环境气溶胶中细菌、真菌和古菌的总量及空间变化等[59–60]。利用核酸片段多样性和梯度凝胶电泳(例如 qPCR-DGGE)可以低成本粗略估计气溶胶中高丰度的微生物类群[61]，但无法精确获得微生物种类信息。从气溶胶样品中获取全部微生物、高质量 DNA 和扩增合适的目标基因是获得气溶胶微生物准确信息的关键。生物气溶胶的宏基因组分析通常采用的目标基因为细菌 16S rRNA 基因高变区(例如 V3–V4)，真菌转录间隔区的高变区(例如 ITS1 和 ITS2)。高通量二代测序技术可以更加全面地揭示生物气溶胶的微生物组，获得比培养方法更高的生物多样性，发现未培养类群和新分类群，揭示气溶胶中微生物的群落构成、传播和变化规律，评估生物气溶胶暴露风险等[5,62–65]。但是二代测序也存在一些缺陷，例如 PCR 扩增过程中易形成嵌合序列导致测序错误，引物偏好性导致优先扩增某些类群，二代测序读长较短致使种或亚种分类元鉴定难度大。纳米孔三代测序的读长比 Illumina 测序的读长更长，可以获得更丰富的微生物属或种水平信息[66]，但是三代测序还需要提高测序精度并降低测序成本。基于气溶胶测序的方法无法判断微生物的生理状态和活性，因此培养和测序方法的结合将更加全面地认识不同环境中生物气溶胶微生物特点和变化规律。

2.2.3 新兴的单细胞水平检测方法

气溶胶颗粒直径分布研究表明，微生物单个细胞可以自由漂浮于空气中[55,67]，这为利用介电泳(dielectrophoresis，DEP)、微流控等技术开展微生物气溶胶单细胞水平分选研究成为可能。如果能够进一步结合一系列新兴的单细胞分析技术，在单细胞水平研究微生物气溶胶不仅有助于全面地解析气溶胶中微生物的组成，还可以实时动态地分析微生物的生理代谢特征。利用介电泳在空间产生不均匀电场使可极化的 *Botrytis cinerea* 、*Pseudoperonospora cubensis* 和 *Podosphaera xanthii* 真菌孢子发生偏移，进而实现温室作物气传病害孢子平均 75%以上的分离和富集效率，有助于温室作物气传病害菌的实时监测[68]。微流控芯片是另一种近年兴起的单细胞分离手段，精准控制微流控芯片内空气动力是气溶胶微生物分离的关键，双平行空气鞘流为空气气流中的微生物颗粒提供稳定的水平动力，然后利用径向空气鞘流为粒子提供侧向平移力，进而实现混合霉菌孢子的分离和富集[69]。微流控芯片与气溶胶毛细管构建的采集装置，能够实现气溶胶颗粒高效的微液滴包裹和 98%以上的收集效率，最后通过染色在 20 s 内区分气溶胶中的微生物和非生物物质[70–71]。虽然这些收集技术取得一定的进展，但是微生物气溶胶单细胞分离技术还不够成熟，污染和设备的小型化等问题亟待解决。

此外，当前的研究主要集中于样品采集，对于后续单细胞水平的检测和表征技术研究较少。期待通过微生物单细胞测序技术弥补宏基因组学水平研究的不足，直接从气溶胶微生物个体基因组水平阐释其种类、生理特性、代谢特点和毒力情况等一系列重要信息。因为测序等侵入性分析方法无法判断气溶胶中微生物是否存活，对微生物气溶胶的致病性会产生较大的误判，无损的单细胞分析方法在微生物气溶胶研究中逐渐引起人们的关注。拉曼光谱是一种产生于分子或晶格振动能级的光子非弹性散射光谱，通过拉曼光谱可以鉴定和分析化学物质，因而被称作物质的“指纹图谱”。拉曼光谱作为一种无损、非接触、非标记和水干扰性小的快速检测技术，在微生物鉴定和表征上已经有大量的应用[72–75]。拉曼光谱技术与气溶胶采集技术的结合为实现气溶胶中微生物的快速捕获和实时检测成为可能。例如由空气收集流和液体收集流构成的两相弯曲管道芯片直接收集空气中微生物，微生物在惯性作用下进入液相并与表面增强材料结合，最后获取其拉曼光谱(图 3A)。Jung 研究团队构建了光流体表面增强拉曼光谱平台，实现了 1 μm 颗粒物 99%的收集效率，100 CFU/mL 的最低检测限[76]。利用单粒子光镊可以在捕获气溶胶化 *Bacillus globigii*，*Yersinia rhodei* 和 *Bacillus subtilis* 等颗粒的同时获得其拉曼光谱，随后通过主成分分析判断气溶胶颗粒中的生物种类[77]。陈建民团队发明了具有表面增强功能的 Klarite 基底(图 3B)，利用该基底可以非培养、快速地检测自助餐厅空气环境中大肠杆菌(*Escherichia coli*)[78]。由于拉曼分析的无损性，分析后的微生物单细胞可以同后续的培养或其他操作无缝对接。同时，可以通过拉曼光谱谱征结合人工智能算法预测细胞是否存活，而不需要通过培养验证，能在极短时间内初步判断微生物气溶胶的危害性，具有很大的优势。拉曼光谱的无损性和指纹表征性适合与微流控等单细胞分离技术联合使用，预期在未来的微生物气溶胶单细胞水平检测中将呈现出更多微生物气溶胶拉曼检测的新技术和新方法。

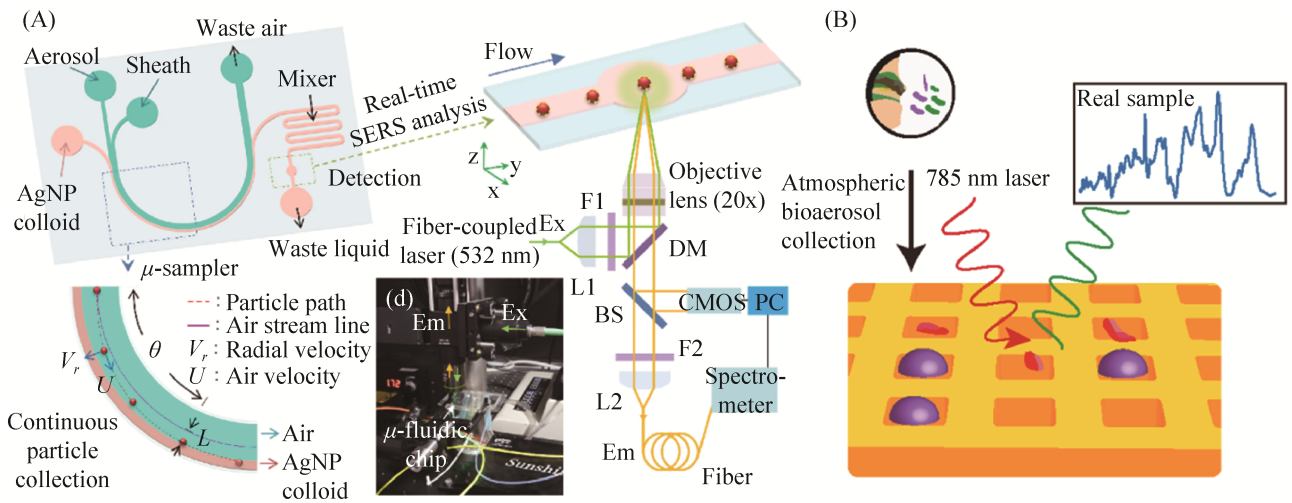

图 3　拉曼光谱检测气溶胶微生物

Figure 3　Schematic diagram of Raman spectroscopy detecting microorganisms in bioaerosols. A: optofluidic SERS platform for continuous characterization of airborne microorganisms[76]; B: Klarite SERS for the rapid detection of *Escherichia coli* in bioaerosols without laborious culture processes[78].

3 气溶胶中病毒的鉴定方法

病毒是微生物气溶胶重要的成分之一，而且SARS、MERS、COVID-19等疫情的传播同病毒气溶胶密切关联。病毒的大小在纳米级别，远远小于细菌的尺寸，相比于细菌气溶胶的收集，病毒气溶胶的采集更有挑战性。由于病毒生命周期的特殊性，无法与细胞形态的微生物一样直接进行培养，而是需要转染特定的细胞系才能判断其活性，过程十分烦琐，因此气溶胶病毒的检测通常是检测其核酸的存在与否。病毒基因组不具有类似16S rRNA基因和18S rRNA基因的通用标记，对于气溶胶中病毒核酸的检测通常采用两种方法。一种是根据特定病毒的基因组设计特异性荧光探针，通过RT-PCR检测。例如，RT-PCR检测A型流感病毒的M基因，B型流感病毒的NS基因表达量来判断学校、医院和养殖场等环境中的病毒量[79–81]。在气溶胶中的病毒种类不明确，没有可用的分子或血清学诊断分析时，通过宏基因组学对空气中病毒进行基因组识别是开发生物检测工具的重要策略[82]。宏基因组分析揭示托儿所空气中人类相关DNA和RNA病毒群落具有季节性变化[83]，为冬季室内病毒传播预防提供重要的科学依据。污水处理厂气溶胶中人类腺病毒、轮状病毒、甲型肝炎病毒和1型单纯疱疹病毒等DNA病毒和少量的*Retroviridae* RNA病毒等也被宏基因组分析发现[84]。需要指出的是，空气中病原体基因浓度低对宏基因组分析带来极大挑战，测序的方法只能显示病毒核酸片段的存在，可能无法提供相关疾病传播和感染性的直接评估。

自2019年新型冠状病毒肺炎暴发以来，新型冠状病毒的扩散、传播与环境的关系受到人们极大的关注。SARS-CoV-2通过飞沫还是气溶胶传播存在较大的争议[85–86]。越来越多的科学家和研究证据表明，新型冠状病毒可以通过气溶胶传播[86–89]，在密闭或拥挤的空间将增加病毒传播的风险。蓝柯团队收集了武汉两家医院的空气样本，通过数字PCR检测SARS-CoV-2的ORF1a/b和N基因，均检测到气溶胶中SARS-CoV-2的RNA[90]，Van Doremalen实验研究证明SARS-CoV-2在气溶胶中的活力和侵染性可以保持数小时，这种活力和侵染性在物体表面甚至可以保持数天，证明了SARS-CoV-2通过气溶胶传播的可能性[91]。类似的感染事件及新型冠状病毒感染家庭的时间和空间分布以及环境变量的研究证明SARS-CoV-2可以通过气溶胶传播[92]。然而，有研究者采用NIOSH旋风采集器收集了普通医疗室以及COVID-19重症监护室护士站和走廊的气溶胶样品，然后通过RT-PCR检测N基因，即使在每平方米8个病毒拷贝的检测极限下仍未检测到新冠病毒核酸[93]。生物气溶胶样品采集的挑战首先是气溶胶中病毒含量非常低，其次是保持病毒颗粒的完整性和活性[94]，然而在气溶胶采集过程中，由于采集器的构造和采集方式等可能损伤病毒颗粒的脂膜或蛋白外壳，进而损害病毒颗粒的活力和侵染性。目前对于新型冠状病毒的生物采集和分析等缺乏统一的标准，因此也缺乏比较性[95]，最终分析结果易受到样品采集条件和检测灵敏性的影响。

快速、便携和灵敏的检测病毒方法对预防和控制病毒的传播至关重要。新技术的交叉有助于气溶胶中病毒的实时、原位、快速和高灵敏检测，例如，将抗体、靶病毒和碱性磷酸酶(alkaline phosphatases, ALPs)设计成三明治结构的表面放大纳米生物传感器可以实现空气中甲型H1N1流感病毒亚PFU/mL检测水平[96]。利用病毒抗原-抗体识别原理结合静电颗粒收集(electrostatic particle concentrator，EPC)可以快速、便捷、特异性地富集气溶胶中的H1N1病毒，检测能力与

qPCR 相当[97]。集气溶胶收集和检测于一体的病毒检测技术将是气溶胶病毒检测的重要发展方向，例如具有气溶胶收集、病毒裂解、RNA 富集和环介导恒温扩增(loop-mediated isothermal amplification，LAMP)功能的病毒检测技术[98]，集气溶胶富集和侧向免疫层析分析(lateral flow immunochromatographic assay，LFA)的检测技术[99]等。目前这些集成采样与检测的实时设备通常可以检测到较高浓度的人工气溶胶病毒，对于气溶胶中载量很低的病毒粒子检测灵敏度还有待进一步提高。

4 展望

自 1833 年查尔斯·达尔文在佛得角群岛首次发现霉菌孢子气溶胶以来，微生物气溶胶领域已经历了将近 300 年的研究历史，近几十年来，微生物气溶胶领域出现了研究热潮，在微生物气溶胶取样和分析技术方面取得了里程碑式的进展。但是，微生物气溶胶的研究方法目前难以全覆盖地收集所有样本，微生物种类鉴定的高不确定性，不同实验室评估误差较大，收集方式对气溶胶中微生物的活性具有不同程度的损害性，可能导致低估了生物气溶胶的风险[100]。首先，推动建立相对统一的生物气溶胶采集器性能评价标准和体系，通过标准和规范的体系降低不同仪器设备参数和收集条件等因素造成的研究结果差异性，进而提高各类研究之间的可比性。其次，建议在研究中采用多种收集器交叉联用，尽可能提高微生物的收集效率和覆盖率。最后，机械应力小、微生物活培养效率高的气溶胶采集装置的研发依然是该领域的重要发展方向。研发微生物气溶胶收集与原位鉴定集成化高灵敏性设备可提升气溶胶病原体快速响应和预警。随着不同学科的交叉，难培养微生物先进培养技术、第三代测序技术、拉曼光谱技术以及人工智能等领域的发展融合，相信微生物气溶胶的研究方法将日新月异。这些新技术新方法也将会大大增加收集与分析的全面性和准确性，加强我们对于微生物气溶胶的微生物组成、活力变化以及传播方式等的深入了解，从而让微生物气溶胶对人类健康的影响降至最低。

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