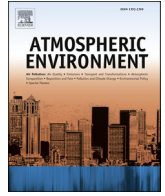




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Non-culturable bioaerosols in indoor settings: Impact on health and molecular approaches for detection



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HIGHLIGHTS

- Non-culturable fraction of bioaerosols is important but still misunderstood.
- Airborne non-culturable agents can be hazardous for human respiratory health.
- Molecular tools allow the detection of airborne non-culturable microorganisms.
- Culture-dependent and independent tools should be used for bioaerosol studies.
- Several research needs relating to non-culturable agents must be addressed.

ARTICLE INFO

Article history:

Received 16 April 2014

Received in revised form

9 March 2015

Accepted 19 March 2015

Available online 20 March 2015

Keywords:

Bioaerosols

Risk assessment

Culture-independent

Molecular methods

Virus

Archaea

Bacteria

Culturable microorganisms

ABSTRACT

Despite their significant impact on respiratory health, bioaerosols in indoor settings remain understudied and misunderstood. Culture techniques, predominantly used for bioaerosol characterisation in the past, allow for the recovery of only a small fraction of the real airborne microbial burden in indoor settings, given the inability of several microorganisms to grow on agar plates. However, with the development of new tools to detect non-culturable environmental microorganisms, the study of bioaerosols has advanced significantly. Most importantly, these techniques have revealed a more complex bioaerosol burden that also includes non-culturable microorganisms, such as archaea and viruses. Nevertheless, air quality specialists and consultants remain reluctant to adopt these new research-developed techniques, given that there are relatively few studies found in the literature, making it difficult to find a point of comparison. Furthermore, it is unclear as to how this new non-culturable data can be used to assess the impact of bioaerosol exposure on human health. This article reviews the literature that describes the non-culturable fraction of bioaerosols, focussing on bacteria, archaea and viruses, and examines its impact on bioaerosol-related diseases. It also outlines available molecular tools for the detection and quantification of these microorganisms and states various research needs in this field.

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1. Introduction

Bioaerosols are airborne particles that contain one or more components of biological origin, typically microorganisms such as bacteria, archaea, fungi or viruses. Bioaerosols are an important transmission route for infectious and sensitization agents, inducing infectious and non-infectious diseases in both animals and humans.

However, there is a substantial lack of information in the field of bioaerosol research in terms of what organisms or components may be found in different environments, how they become aerosolised, and their impacts on health. Most studies have focused on occupational bioaerosol-related hazards faced by workers who are continually exposed to highly contaminated environments.

Bioaerosols have traditionally been studied using culture methods (Duchaine et al., 1999, 2000; Dutil et al., 2009; Meriaux et al., 2006b), which allow scientists to recover only culturable organisms, using prescribed growth media and specific conditions. However, only a small proportion of the total bioaerosol burden in any given environment is culturable. There are two main

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explanations for this: firstly, there are many biological components that cannot be measured by culture, such as cellular fragments and components; and secondly, many organisms are not easily cultured or are rendered unculturable by air sampling or aerosolization processes (Amann et al., 1995; Peccia and Hernandez, 2006). Whilst these non-culturable agents may not be infectious, they can cause inflammation and sensitization (Girard et al., 2009; Glazer et al., 2007; Thorne et al., 2006), and they can exacerbate existing chronic respiratory diseases.

Over the past ten years, studies using molecular methods for the detection of airborne microorganisms have revealed that non-culturable microorganisms are considerable constituents of bioaerosols. The importance of the application of these methods was illustrated when they were used in the discovery of significant amounts of archaea present in the atmospheres of several working environments, in particular in agricultural settings (Blais Lecours et al., 2012; Just et al., 2013; Nehme et al., 2009). Archaea are fastidious organisms (Khelaifa et al., 2013; Wolfe, 2011), and consequently, traditional techniques had never identified them in bioaerosol studies. Newly described bioaerosol components such as these could be a factor in several bioaerosol-related diseases with unknown aetiologies (Eduard and Heederik, 1998). For example, chronic bronchitis is prevalent in people who work in swine barns (Sahlander et al., 2012), but the definitive cause has yet to be clearly identified. It is therefore important to employ several detection approaches, including non-culturable testing.

Although moulds are an important component of bioaerosols (Zukiewicz-Sobczak, 2013) and, in some cases, can be included in the non-culturable fraction of bioaerosols, they will not be discussed in this review. Their conidia and spore structures are designed to allow airborne spreading, and thus are well adapted for transport through air currents. Additionally, they are not as sensitive to aerosolization and sampling stresses as bacteria, archaea or viruses and are easier to detect by culture-dependent methods. Fungal spore counts and fungal fragment evaluations are often used for their detection and few molecular approaches have been developed for their detection in bioaerosols (Gorny, 2004; Lee and Liao, 2014; Simon and Duquenne, 2014).

This review focuses on non-culturable agents, including bacteria, archaea and viruses, which are found in bioaerosols of indoor environments, and their potential impact on human health. We describe the molecular tools that can be used to detect non-culturable agents and also highlight the importance of combining culture-dependant and culture-independent approaches for thorough bioaerosol characterization. This review also states some research needs that must be addressed in the future.

2. Bioaerosol-related diseases

2.1. The impacts of bioaerosols on human health

Bioaerosols can affect human health in many ways. When carrying and transmitting infectious microorganisms, bioaerosols may initiate an infection in the respiratory tract or other parts of the body (Roy and Milton, 2004; Roy and Reed, 2012). Well known examples of airborne respiratory diseases include tuberculosis (LoBue et al., 2010), the common cold (Jaakkola and Heinonen, 1995), influenza (Bridges et al., 2003), and legionellosis (Nguyen et al., 2006). Only viable airborne agents can cause infectious diseases. In addition, bioaerosols that contain non-culturable microorganisms or their fragments can lead to chronic or acute diseases (Bunger et al., 2004; Fogelmark et al., 1991; Shahan et al., 1994). These non-infectious bioaerosol components can have immunogenic potential that induce sensitization diseases if they are present in sufficiently high concentrations (Falkinham, 2003). It is well

documented that humans who are exposed daily to these airborne particles can develop allergic and chronic inflammatory responses (Eduard, 1997; Poole and Romberger, 2012). The impact of the inhalation of bioaerosol components on human health depends on various factors such as their infectivity, airborne concentration, immunogenicity and particle size.

Both the upper and lower respiratory tracts are exposed to bioaerosols. Diseases typically manifest in a particular part of the respiratory tract. Viruses are the main cause of infections in the upper respiratory tract (sinusitis, pharyngitis) (Lopardo et al., 2012), including seasonal influenza and viruses that cause the common cold (rhinovirus, adenovirus, coronavirus) (Louie et al., 2005). Sensitization diseases in the upper airways include allergic rhinitis and sinusitis and can be caused by whole or fragmented organisms (allergens, endotoxins, peptidoglycan and bacterial DNA) (May et al., 2012). On the other hand, lower respiratory tract diseases (bronchitis and pneumonia) are predominantly caused by bacteria (e.g. *Legionella* spp., *Streptococcus* spp., *Haemophilus influenzae*) (Dasaraju and Liu, 1996), although avian influenza (H5N1) can also infect the lower respiratory tract, along with parainfluenza viruses and respiratory syncytial viruses (Hall, 2001). This is also the site of chronic diseases such as asthma (bronchus) (Sferrazza Papa et al., 2014), hypersensitivity pneumonitis (alveolar) (Takemura et al., 2008) and chronic obstructive pulmonary diseases (bronchus) (Bettoncelli et al., 2014), which can be caused by bacterial and fungal cells and spores (O'Connor et al., 2013), allergens, peptidoglycan, and endotoxins (Reynolds et al., 2013), which are present on non-culturable agents.

2.2. Bioaerosols-related diseases in indoor settings

Bioaerosols are ubiquitous, but they are especially present in indoor environments due to the lack of ventilation and dispersal mechanisms. Indoor settings that contain particularly high concentrations of bioaerosols pose a greater risk to human health than facilities harbouring low airborne biological contaminants (May et al., 2012). Certain indoor settings such as agricultural settings, schools and homes may allow the accumulation of high bioaerosol concentrations, given the density of potential bioaerosol sources (humans, animals, plants) and the lack of efficient ventilation or air exchange systems (Table 1). People exposed to such indoor conditions are thus at risk of developing a wide range of respiratory and non-respiratory diseases and conditions (ATS, 1998; Douwes et al., 2003; Kirkhorn and Garry, 2000; Linaker and Smedley, 2002; National Institute for Occupational Safety and Health, 2007; van Kampen et al., 2012). These diseases are often not attributed to specific etiologic agents since a wide variety of organic products (such as allergens) can cause similar symptoms, and it is difficult to identify a specific cause.

2.3. The difficulty to correlate a disease with a bioaerosol component

For several indoor settings, including industrial facilities, domestic environments and medical clinics, information on total airborne bacteria, total airborne archaea, and airborne viruses is incomplete (Table 1), which prevents an overall knowledge and understanding of the total biological burden of these facilities. It is likely that the unknown non-culturable fraction of bioaerosols, which can be found only by culture-independent methods, plays an unsuspected role in bioaerosol-related diseases. With a continuing increase in the frequency of studies characterizing the non-culturable fraction of bioaerosols, shown in Fig. 1, the role this fraction plays on human health will become clearer.

Moreover, the correlation between the presence of traditionally

Table 1
Non-culturable quantification of total airborne bacteria and archaea in indoor environments by molecular approaches compared to culture counts (N/A: Not available).

Level of contamination	Indoor setting	Culturable counts (CFU/m ³)		Non-culturable counts (16 S/m ³)	
		Bacteria		Bacteria	Archaea
High	Swine barns	10 ⁵ (Chang et al., 2001; Nehme et al., 2008), 10 ⁴ to 10 ⁸ (Lee et al., 2006b; Verreault et al., 2010)		10 ⁷ to 10 ⁸ (Nehme et al., 2008), 10 ⁷ (Hong et al., 2012), 10 ⁶ to 10 ⁸ (Verreault et al., 2010)	10 ⁶ to 10 ⁸ (Nehme et al., 2009)
	Dairy barns	10 ⁴ to 10 ⁶ (Duchaine et al., 1999), 10 ⁴ (Lee et al., 2006b), 10 ⁴ to 10 ⁵ (Abd-Elall et al., 2009), 10 ⁴ (Alvarado et al., 2009), 10 ⁵ (Kullman et al., 1998)		10 ⁸ (Blais Lecours et al., 2012)	10 ⁵ (Blais Lecours et al., 2012)
	Poultry and turkey barns	10 ¹ to 10 ⁶ (Martin and Jackel, 2011), 10 ³ (Oppliger et al., 2008), 10 ² to 10 ⁶ (Lawniczek-Walczyk et al., 2013), 10 ⁵ (Fallschissel et al., 2010)		10 ³ (Nonnenmann et al., 2010), 10 ⁸ (Oppliger et al., 2008)	10 ⁴ to 10 ⁶ (Just et al., 2013)
	Peat moss factories	10 ³ to 10 ³ (Meriaux et al., 2006a; Meriaux et al., 2006b)		N/A	N/A
	Wastewater treatment plants	10 ³ to 10 ⁴ (Bauer et al., 2002), 10 ¹ to 10 ⁵ (Laitinen et al., 1994), 0 to 10 ³ (Lee et al., 2006a), 10 ² to 10 ³ (Li et al., 2013)		10 ⁷ to 10 ⁸ (Blais Lecours et al., 2014)	10 ⁴ (Blais Lecours et al., 2014)
	Composting facilities	10 ³ to 10 ⁴ (Bru-Adan et al., 2009), 10 ⁵ to 10 ⁷ (Albrecht et al., 2007)		10 ⁵ to 10 ⁶ (Bru-Adan et al., 2009)	N/A
Moderate	Vegetable/seed processing	10 ⁴ to 10 ⁶ (Lee et al., 2006b), 10 ¹ to 10 ⁵ (Gora et al., 2009)		N/A	N/A
	Machining industries	10 ¹ to 10 ³ (Gilbert et al., 2010c), 10 ¹ to 10 ⁴ (Park et al., 2010), 10 ¹ to 10 ² (Gorny et al., 2004)		N/A	N/A
	Forest product industries	10 ² to 10 ⁴ (Park et al., 2010), 10 ² to 10 ⁵ (Duchaine et al., 2000)		N/A	N/A
	Subway station	0 to 10 ³ (Dybwad et al., 2014), 10 ¹ to 10 ² (Kim et al., 2011)		N/A	N/A
Low	Schools and daycares	10 ¹ to 10 ³ (Lignell et al., 2005)		N/A	N/A
	Homes	10 ² to 10 ³ (Frankel et al., 2012), 10 ³ (Bouillard et al., 2006)		Biodiversity (Adams et al., 2014)	N/A
	Offices	10 ¹ to 10 ³ (Golofit-Szymczak and Gorny, 2010), 10 ² (Tsai and Macher, 2005), 10 ⁰ to 10 ³ (Bholah and Subratty, 2002), 10 ¹ to 10 ² (Bonetta et al., 2010), 10 ¹ to 10 ³ (Bouillard et al., 2005), 10 ¹ to 10 ² (Tham and Zuraimi, 2005), 10 ³ to 10 ⁴ (Lis et al., 2004), 10 ² to 10 ³ (Sessa et al., 2002), 10 ¹ to 10 ² (Reynolds et al., 2001)		Biodiversity (Gaüzère et al., 2014)	N/A
	Dentist offices	10 ⁰ to 10 ⁴ (Dutil et al., 2009), 10 ¹ to 10 ² (Hallier et al., 2010)		N/A	N/A
	Hospitals	10 ¹ to 10 ² (Gilbert et al., 2010a), 10 ² to 10 ³ (Matouskova and Holy, 2013), 10 ¹ to 10 ³ (Park et al., 2013)		Biodiversity (Gaüzère et al., 2014)	N/A

measured airborne components and diseases is not always consistent, in that high exposure of these components does not always lead to higher risk of developing diseases (Poole and Romberger, 2012; Zhiping et al., 1996). It is known that the thermophilic actinomycete *Saccharopolyspora rectivirgula* can induce hypersensitivity pneumonitis (farmer's lung) and that exposure to

endotoxins is responsible for organic dust toxic syndrome, but are other undetected components also involved in the development of those diseases? Is there a synergy between various components of bioaerosols to exacerbate certain diseases, or even to protect the host from some diseases? It has been hypothesized that hypersensitivity pneumonitis could be developed after infection by the influenza virus (Cormier et al., 1994, 1993; Dakhama et al., 1999). The interaction of various airborne components and their impact on human health is not well known, and is an area that needs to be further explored.

3. Non-culturable bioaerosol components and their impact on respiratory diseases

For a long time, scientists have tried to determine the aetiology of bioaerosol-related diseases by examining airborne biological components in various environments, often without clear outcomes. Endotoxins remain the main non-culturable agent in bioaerosols that are tested for during bioaerosol-exposure assessment studies (Dosman et al., 2006; Rylander and Carvalho, 2006; Zejda et al., 1994). Advances in technology now allow scientists the ability to measure non-culturable biological components in the bioaerosols of many indoor environments (Blais Lecours et al., 2012; Eduard et al., 2012; Nehme et al., 2008; Oppliger et al., 2011). However, knowledge remains incomplete, with only a few teams routinely searching for viruses (Corzo et al., 2013; Myatt et al., 2004; Verreault et al., 2010) and archaea (Blais Lecours et al., 2012; Just et al., 2013; Nehme et al., 2009) in bioaerosol characterization studies. The next section of the article reviews the main non-culturable agents that should be looked for in exposure assessment studies, and their known and potential effects on human health.

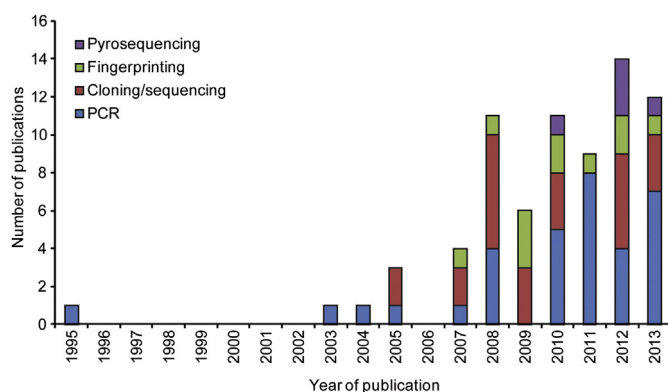


Fig. 1. Publications using molecular methods to characterize bioaerosols of indoor environments. In order to find articles that had used molecular methods to detect microorganisms in bioaerosols, a literature search was performed using Web of Science™. Four search phrases were used to conduct the search in two fields: in the topic field 'indoor OR agriculture* OR environment' AND 'microorganism* OR biologic* OR bacteria* OR virus* OR archaea*' in the topic; and in the title field 'bioaerosol* OR airborne' NOT 'performance OR evaluation OR inactivation'. Over 750 articles were returned, so the non-relevant articles were removed individually. Only the articles that had described the bioaerosols of an indoor environment using a molecular method were included in the graph. The final number of articles was 60. Methods were divided into PCR, cloning/sequencing, fingerprinting, and pyrosequencing, and plotted in a graph against their year of publication.

3.1. Archaea

Methanogens, a subgroup of strict anaerobes in the archaeal phylum *Euryarchaeota*, are the perfect example of agents found in high concentrations in various work-related bioaerosols (Blais Lecours et al., 2014; Blais Lecours et al., 2012; Just et al., 2013; Nehme et al., 2009), which have never been cultured from air samples. Archaea are microorganisms constituting one of the three domains of life, along with *Eukarya* and *Bacteria*. Archaea are prokaryotes, like bacteria, and both domains share characteristics in their morphology and metabolism. However, archaea have unique characteristics that distinguish them from bacteria: their unique membrane lipids (Kates, 1992; Sprott et al., 2003); a cell wall devoid of peptidoglycan (Kandler and König, 1998); the intrinsic capacity to resist several antibiotics (Dridi et al., 2011; Kandler and König, 1998); different ribosomal 16S DNA (Baker et al., 2003; Brochier-Armanet et al., 2011; Pester et al., 2011; Woese et al., 1990); and their genetic processes which resemble those of eukaryotes (Allers and Mevarech, 2005; Forterre et al., 2002; Schleper et al., 2005). Archaea are resilient microorganisms that thrive in extreme environments, and are found in complex microbial communities such as the gut (Nottingham and Hungate, 1968), and in swine and cow manure (Gattinger et al., 2007; Snell-Castro et al., 2005; Whitehead and Cotta, 1999). It is now known that archaea are also an important part of the aerial environment. As hypothesised by our team in 2008, airborne archaea were detected in high concentrations in bioaerosols of various working environments such as agricultural settings (Blais Lecours et al., 2012; Just et al., 2013; Nehme et al., 2009) and wastewater treatment plants (Blais Lecours et al., 2014), using non-culture techniques. A recent study has shown that methanogens can induce chronic inflammation in the lungs, which can lead to sensitization diseases (Blais Lecours et al., 2011).

3.2. Viruses

Viruses that can be spread by the aerosol route constitute a significant fraction of transmissible infections. Given their complex culture requirements, they are excellent examples of agents in bioaerosols that are difficult to culture. Some examples of airborne-transmitted viral diseases are influenza A and B (flu), coronaviruses (common cold and severe acute respiratory syndrome), adenoviruses (common cold and lung infections), norovirus (gastrointestinal illnesses) and morbillivirus (mumps and measles). Some viruses can also induce an inflammatory response when present in an inactivated or non-replicating form: inactivated Respiratory Syncytial Virus (RSV) was shown to induce a response by macrophage inflammatory proteins in epithelial cells (Harrison et al., 1999). However, given their importance in airborne disease transmission and, presumably, the airborne microbial community, airborne viruses are markedly absent in studies that describe the content of bioaerosols. This can be largely attributed to the lack of efficient culture techniques. In the last ten years, the number of studies investigating airborne viruses has increased, however there are still difficulties with the recovery and sampling of airborne viruses. There is no universal assay for viruses, however some publications have reported universal PCR assays for groups of viruses, such as an assay that can amplify RNA from all three flavivirus subgroups (Maher-Sturgess et al., 2008), and another that amplifies RNA from all influenza A viruses (Hoffmann et al., 2001). A neuraminidase assay was described as a potential broader marker for the presence of certain viruses (Turgeon et al., 2011; Zhang et al., 2012). Whilst such assays may be useful for detection of suspected viruses in clinical samples, it is difficult to use in indoor environmental settings where the airborne biomass is unknown. Therefore the best approach would be to conduct preliminary

studies to describe airborne viruses, in order to determine the viruses that are prevalent in specific environments. One such study was conducted in a solid waste treatment plant, where they used both non-culture techniques and culture techniques to determine which viruses were present in the bioaerosols (Carducci et al., 2013).

3.3. Mycobacteria

Non-tuberculous mycobacteria (NTM) are found in many water-related sources, soil, and metal-working fluids (Cayer et al., 2007; Falkinham, 2009). They are difficult to culture, with slow-growing colonies taking 2–3 weeks to appear, however, recently molecular biology tools for the detection of NTM have been developed (Veillette et al., 2008, 2004). NTM are an example of microorganisms that can cause disease in the lungs whether or not they are infectious. Mycobacterial cells and their components are known to cause inflammation in the lungs (Falkinham, 2003). Virulence factors of NTM are unknown (Falkinham, 2009); however it is known that exposure to bioaerosols containing NTM can be followed by hypersensitivity pneumonitis (HP) (Marras et al., 2005). One study showed that, in metal-working fluid facilities where workers had HP, there was a significantly higher amount of NTM in the fluid compared to facilities where no HP had been diagnosed amongst workers (Shelton et al., 1999). Another study showed that several species of NTM induced inflammatory responses in mouse macrophage cells (Huttunen et al., 2000). Naturally occurring *Mycobacterium tuberculosis* in aerosols can be viable but may not be culturable (Schafer et al., 1999). Several studies have characterized airborne mycobacteria in indoor settings using culture-independent methods, such as peat moss processing plants (Cayer et al., 2007), dental units (Dutil et al., 2007), closed environments containing therapy pools and hot tubs (Glazer et al., 2007) and even hospitals (Vadrot et al., 2004).

3.4. Gram-positive bacteria: culturable but rarely tested for

Gram-positive bacteria, especially anaerobes, may be culturable after aerosolization, but are not often considered in culture-based bioaerosol characterisation studies (Gora et al., 2009). Recently, molecular methods have shown that faecal anaerobes are major components of bioaerosols in various environments, such as agricultural settings (Gora et al., 2009; Nehme et al., 2008). Their potential impact on respiratory health is often underestimated, with many studies instead focussing on Gram-negative bacteria due to their lipopolysaccharides (endotoxins), a well-described component of bioaerosols, known to be an aetiological factor of several bioaerosol-related diseases (Bouillard et al., 2005; Lawniczek-Walczuk et al., 2013; Zejda et al., 1994).

4. Molecular tools for detection and quantification of non-culturable airborne bacteria, archaea and viruses

This section of the review describes tools available for the detection of non-culturable airborne agents. Essentially, all existing methods for environmental samples can be applied to air samples. In this review we focus on the most promising methods for future development. Although imaging methods can be used, the most promising methods available are genomic, with databases currently in development.

Genomic methods exploit the presence of nucleic acids of target microorganisms in any given sample, and are not influenced by the culturability of an organism. There is a wide variety of genomic methods available, each with its own advantages and disadvantages.

Quantitative polymerase chain reaction (qPCR) is an excellent tool used to detect and quantify microorganisms from bioaerosol samples (Peccia and Hernandez, 2006). qPCR has progressed significantly over the past decade, as has its application to air sampling. Prior to its development, endpoint PCR was used to analyse air samples (Alvarez et al., 1994; Mukoda et al., 1994). However this method only allows the user to determine whether or not a target organism is present, and does not provide relevant quantitative information. It is therefore not commonly used for air sampling, unless one aims to simply detect a certain agent. An excellent advantage of qPCR is its ability to quantify the amount of genomic material in a sample. This is very important since the concentration of bioaerosols is an important factor in determining the impact of bioaerosols on human health. Consequently, we may be able to estimate the level of risk that an environment poses using qPCR.

PCR can be used to analyse bioaerosol samples for either groups of microorganisms (e.g. total bacteria) or specific organisms. Total bacterial and archaeal data are very useful in the evaluation of the airborne contamination of a given environment, because they allow us to target all species present in the sample. Although primers can have more affinity towards some organisms which can lead to some biases (Suzuki and Giovannoni, 1996), this type of test has allowed for the detection of organisms that were previously unknown to be airborne contaminants (Nehme et al., 2009). Unfortunately, no universal PCR assay exists for the detection of viruses (Prussin et al., 2014; Turgeon et al., 2011), so investigators must target specific viruses (Carducci et al., 2013; Masclaux et al., 2014; Verreault et al., 2010). This can lead to the omission of unsuspected viruses, and subsequent underestimation of the diversity of airborne viruses in the studied environment. Carducci et al. suggested that 'index' viruses could be targeted in initial studies (Carducci et al., 2013), but that it would be important to select viruses appropriate to the environment being tested. In their study on solid waste treatment plants, tests were included for hepatitis (types A, B and C), norovirus, adenovirus, and rotavirus (Carducci et al., 2013).

The use of PCR in bioaerosol characterization studies has increased greatly in recent years (Fig. 1). Protocols that are commonly used in bioaerosol studies include the 16S rRNA gene for total bacteria (Blais Lecours et al., 2012; Masclaux et al., 2013; Nehme et al., 2008; Oppliger et al., 2008), total archaea (Blais Lecours et al., 2012), influenza (Perrott et al., 2009), *Saccharopolyspora rectivirgula* (Blais Lecours et al., 2012) and bacteriophages (Verreault et al., 2011).

In the past, many researchers were reluctant to accept the application of PCR to bioaerosol sampling because of its inability to distinguish between viable and non-viable microorganisms. However, a recently developed tool allows for the discrimination between intact and non-intact microorganisms, which is a strong indication of whether or not microorganisms are viable. It also allows the exclusion of 'free' genetic material that is present outside of cells in the sample. Propidium monoazide (PMA), which was developed from propidium iodide (PI), is a membrane-impermeable molecule that has been used extensively in fluorescence microscopy to discriminate between viable and non-viable cells, as it generally only permeates cells that have a disrupted cell membrane. It was modified by adding an azide group that binds to DNA upon exposure to light, rendering the DNA unavailable for synthesis, i.e. amplification in PCR (Nocker et al., 2006). Theoretically it should also bind to extraneous nucleic acids, which can give inflated results. PMA has been used in conjunction with qPCR to determine the differences between viable (intact) and non-viable (damaged/free DNA) cell counts in some water and environmental samples (Parshionkar et al., 2010), and, recently, has also been applied to air samples (Bonifait et al., 2014; Kaushik and

Balasubramanian, 2013; Vesper et al., 2008).

Metagenomic approaches are used to directly characterize the total genetic components of a bioaerosol sample using, amongst others, pyrosequencing and next generation sequencing platforms (e.g. Illumina MiSeq and LifeTechnologies Ion Torrent Personal Genome Machine (PGM)) (Chen and Pachter, 2005; Leung et al., 2014; Meadow et al., 2014). Recently, these methods have become more affordable and accessible to a wide population of scientists, and it is anticipated that information on bioaerosol metagenomes will subsequently increase significantly. In various environmental studies, including bioaerosol studies, pyrosequencing has identified many organisms that were previously undetected, and has been used to identify airborne metagenomes (Tringe et al., 2008). These approaches could be interesting tools for relating gene functions of microorganisms to bioaerosol-related diseases.

Various techniques that can be used to characterize the total biodiversity of a bioaerosol sample and evaluate the possible aetiological agents of bioaerosol-related diseases include cloning/sequencing, fingerprinting, pyrosequencing, and next-generation sequencing platforms, all performed on 16S rRNA genes or ribosomal RNA gene spacers. Cloning/sequencing techniques have been used in some bioaerosol studies (Cayer et al., 2007; Nehme et al., 2009) and allow very clear identification of species present in a sample. These techniques are time-consuming, however their costs have recently significantly reduced. Fingerprinting methods such as DGGE, TGGE, T-RFLP and ARISA are other techniques used for biodiversity studies (Blais Lecours et al., 2012; Gandolfi et al., 2013; Gilbert et al., 2010b; Maron et al., 2005). These are particularly useful in longitudinal studies aiming to evaluate a change in the complex microbial communities over time. Finally, pyrosequencing and next-generation sequencing methods allows identification of all targeted microorganisms in a sample without significant bias (Nonnenmann et al., 2010), however the associated bioinformatics require time and expertise. Nevertheless, these techniques are very promising as they can be used to discover underrepresented microorganisms from an environment that may be implicated in bioaerosol-related diseases.

Whilst genomic methods are an excellent tool in bioaerosol characterization studies, there are two major problems. The first is the bias introduced by the method chosen for nucleic acid extraction, as the yield and the biodiversity of the microorganisms detected will depend on the extraction protocol being used (Morgan et al., 2010). Recent literature strongly suggests the inclusion of a mechanical cell break-up in the extraction protocol (Morgan et al., 2010). The other problem is that according to the rare biosphere theory, only the dominating sequences are detected when using molecular approaches (e.g. pyrosequencing) (Shade et al., 2012). Bioaerosol biodiversity can therefore be underestimated, making it more difficult to link bioaerosol components to the diseases. However, with careful selection and optimisation of the most appropriate techniques, and continued development of databases, these tools have great potential and have so far allowed for the discovery of important new information about bioaerosol diversity.

5. Complementarity of culture and culture-independent analyses

In bioaerosol characterisation studies, it is important to ensure a complete picture is formed as to what is present in the air, and whether it has the potential to cause infectious diseases, or allergic and hypersensitivity diseases. The best way to achieve this is to employ a complementary set of methods that include both culture-dependent and culture-independent techniques (Shade

et al., 2012). This would help to reduce the bias introduced by the use of only one type of method, and give a more complete set of data that allows the detection of microorganisms that are systematically underestimated by either method. A culture technique may allow for the characterisation of microorganisms present in low concentrations, which are clinically important, such as Gram-negative bacteria. On the other hand, culture-independent methods including molecular approaches will identify the microorganisms or cellular components that are present in high concentrations, yet often overlooked by culture methods, such as anaerobic Gram-positive bacteria. This has been performed in several studies, particularly in the simultaneous use of PCR and culture for air samples (Hubad and Lapanje, 2013; Shade et al., 2012).

6. Research needs

6.1. Knowledge gaps in the literature concerning non-culturable microorganisms

Extensive qualitative description of bioaerosol components in numerous indoor environments is necessary in order to better understand their health impacts on humans. The knowledge gap of non-culturable data fed a vicious cycle whereby researchers chose to target only culturable organisms. However, with increasing use of non-culture techniques in bioaerosol research, as demonstrated in Fig. 1, researchers are becoming more confident in choosing to use non-culture techniques, which will lead to a narrowing of this significant knowledge gap.

Another issue in this field is the difficulty involved in knowledge translation from fundamental to clinical sciences. Bioaerosol science is very multidisciplinary, and the importance or perspective in data interpretation can change depending on the scientific background of the researchers. For example, microbiologists are interested in the microbiological content of bioaerosols; bioinformaticians use genomics to develop tools for genomic analysis; physicists focus on modelling; and physicians are concerned with diseases. It is essential that experts from each of these disciplines work together to obtain a complete picture of bioaerosol risk assessment.

6.2. Longitudinal follow-up studies

Seasonal variation of non-culturable biodiversity is poorly understood (Carducci et al., 2013; Nehme et al., 2008), whereas it has often been investigated in culture-dependent studies (Dybwad et al., 2014; Mentese et al., 2012; Wang-Li et al., 2013). Longitudinal follow-up using molecular biology approaches is necessary to give a complete picture of the bioaerosol burden over a time frame. Indeed, several seasonal factors can influence the aerosolisation of microorganisms, such as humidity and temperature, which fluctuate between seasons (Frankel et al., 2012; Nehme et al., 2008).

6.3. Exposure limit values and risk assessment

An important problem in indoor settings contaminated with bioaerosols is the lack of exposure limit values (ELV) established for their various components. The few existing ELV have been set using culture detection data, which do not take into account the non-culturable microorganisms. The lack of appropriate ELVs can be attributed to several factors, including: the lack of studies on non-culturable bioaerosols; the lack of longitudinal variation of bioaerosols' concentration and biodiversity; a poor knowledge of dose–response effects of airborne components; and the difficulty to conduct meticulous epidemiological studies (Goyer et al., 2001).

To determine appropriate ELVs, it will be necessary to estimate human exposure to bioaerosols in indoor settings, and to study and determine the normal airborne background (culturable and non-culturable microorganisms) of these environments.

The control banding method is a widely employed risk exposure assessment method in Europe, but is mostly used for chemicals (Chalupka, 2010; Zalk and Heussen, 2011). However, it has been proposed as a tool to assist in controlling human exposure to hazardous bioaerosol components in some indoor environments (Cohen and White, 2006; Lavoie et al., 2013). This method gives bands of hazards ranging from high to low, according to the toxicity or severity of the material and the probability of exposure. However, this method does not take into consideration the allergenic fraction of bioaerosols, which is a significant hazard in indoor settings. If control banding could be adapted to incorporate all bioaerosol components, it would be a useful tool for estimating the exposure risks of bioaerosols to humans.

7. Conclusion

Large knowledge gaps remain in the field of bioaerosol science, despite recent developments. Due to its multidisciplinary nature, there is a distinct lack of translation between different types of data, which leads to a disjointed understanding in bioaerosol diversity, and the effects of bioaerosols on human and animal health. In particular, this review reveals a paucity of studies routinely investigating the presence of viruses and archaea in bioaerosols, which are clinically significant components of bioaerosols. If we are to have a better overall understanding of bioaerosols, particularly in indoor environments, it is necessary to conduct studies that characterize the entire population of bioaerosol communities, which requires careful selection of a range of detection methods. It will also be necessary to be able to interpret and translate this data across the various disciplines involved in bioaerosol research in order to better understand the impact of bioaerosols on human health.

Acknowledgements

P.B.L. is a CIHR Strategic Training Fellow in PHARE and received a CIHR-Quebec Respiratory Health Training Program short-term training scholarship. C.D. is a member of the FRQ-S Respiratory Health Network, and a FRQ-S Senior Scholar. We thank Luc Trudel for his comments and insights and Judith-Elise Marcoux for English revision.

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