

Measuring environmental fungal exposure

E. R. TOVEY & B. J. GREEN

Department of Medicine, University of Sydney, NSW, Australia and Woolcock Institute of Medical Research, Sydney, NSW, Australia

Airborne fungi are ubiquitous in the environment and human exposure is inevitable. Such fungi differ greatly in their taxonomic, physical, ecological, behavioral, and pathogenic characteristics. Many strategies have evolved to sample, identify and interpret fungal exposure and their choice is determined by the hypotheses involved. While fungi can be sampled directly from surfaces, results do not generally reflect human exposure. For this reason, airborne spores are commonly sampled, by either filtration or impaction, using volumetric air samplers. Identification is commonly performed by either culture on nutrient medium or light microscopy using morphological criteria, although new techniques using DNA probes or characteristic antigens or toxins continue to be developed. Interpretation of such exposure data is both complex and contentious, but while there are numerous recommendations there is no consensus on exposure thresholds. A better understanding of the complex pathogenic roles of fungi and susceptibilities of their hosts will enable refinement of techniques for sampling and interpretation.

Keywords air sampling, conidia, fungi, spores

Introduction

Fungi are a diverse lineage of ubiquitous eukaryotic micro-organisms. They grow as saprophytes on non-living organic matter or function as invasive or symbiotic organisms in living tissue where their thread-like hyphae form mycelial networks. Approximately 69,000 species have been identified and up to 1.5 million species may exist [1]. In some species of agricultural, food, or medical interest, over 100 different individual stains are recognized, including *Aspergillus fumigatus*.

The principal dispersal vectors of fungi are sexual spores or asexual conidia. These vary in size (2–70 µm) and shape (spheres, rods, chains, etc.), depending on the species. Aerosolized viable hyphae may also disperse the organisms, although they lack the morphological characteristics to be directly identified. Exposure to moulds, both indoors and outdoors, is

inevitable, and airborne concentrations in the range of 10^1 – 10^5 spores/m³ of air are common [2].

Methods applied to estimate exposure to fungi must balance the level of information required to answer specific questions within the numerous practical and technical limitations of the techniques that have been developed. There are three phases to measuring environmental exposure: sampling, identification and interpretation.

Sampling

For simple measurement of mould presence, mycelia can be directly sampled from surfaces by swabbing or by lifting with an adhesive tape. Such samples are then generally subjected to either culture or direct microscopy [3]. The principle advantages are speed, simplicity, and the high recovery of organisms available for analysis. The main disadvantage is the lack of quantitative correlation of these samples with both the number and identity of airborne organisms a person may be exposed to, and thus any interpretation in terms of relevance to disease pathology may be limited [4].

Correspondence: E. Tovey, Rm 461, Blackburn Building, DO6, University of Sydney, NSW 2006, Australia. Tel: +61 2 9351 2093; Fax: +61 2 9351 7451; E-mail: ert@mail.med.usyd.edu.au

The measurement of ambient mould spores requires their recovery and isolation from a volume of air. Not only is this more representative of the airborne exposure of people, but in many circumstances there are no appropriate surfaces available to represent multiple and distant sources. Many different designs of the devices used to collect air samples have evolved to match the different characteristics of the organisms and the forms of analysis to be applied. These devices allow various sized particles to be settled, impacted, filtered or impinged onto collection agents such as porous filters, agar media, adhesive films, or into nutrient liquids. The collection efficiency for the various sizes and shapes of spores differs widely due to the interaction between the characteristics of particles (particularly drag and inertia) and the sampler's design and use (inlet shape, face velocity, and the orientation relative to air currents, etc.) [5]. In addition, some spores behave less predictably due to the effects of their non-spherical shape and surface ornamentation. Such mechanical sampling devices partly imitate the complex series of events occurring during human exposure to spores. Here, inhalation via the nose occurs through two small downward-facing orifices with oscillating airflow and the spores are collected at different sites within the airways by a combination of impingement, adhesion and impaction.

Sampling strategies need to reflect the many different ecological variables involved in exposure, where spore concentration differs in space, time and in the mixture of species present. At one end of the spectrum are immobile (static) samplers used in a fixed location to represent ambient concentration over an area, while at the other end are small personal samplers, worn on the body to represent an individual's local exposure. The ambient concentration of spores can fluctuate by several magnitudes over a period of time – minutes, hours, or days – depending on the situation. The time used for collection also varies, from 'grab' samples collected over seconds or minutes (Anderson-type) to samplers operating independently for up to a week (Hirst-type). The choice of samplers reflects both the limitations of the analysis (e.g., long sampling times could overload culture plates) as well as the need for temporal information. Thus, any sample only provides an approximation of the exposure an individual or a community may receive over time in that location. Such a strategy may need to perform repeated sampling over the course of days and use multiple samplers. Our group has developed small filters that are worn inside the nostrils to collect inhaled particles. These provide a unique insight into personal

exposure to spores over approximately 5 µm in diameter [6].

Identification

Numerous methods have been developed to identify the collected fungi. The two most common methods, which require specialist identification skills, are direct histochemical staining followed by microscopic visualization of spore morphology, and secondly, culture of viable spores on specific nutrient media. More refined techniques, more commonly used to differentiate between strains of a species, involve the production of characteristic proteins or toxins, fine surface morphology resolved by scanning electron microscopy, or the development of specific molecular probes. While culture may provide great discrimination, it has disadvantages: it is slow, it can be critically influenced by the nutrient medium chosen, non-viable species are not detected, and competition and inhibition from other colonies may inhibit growth. Fungi may also be quantified by analysis of specific component molecules, for example, by RT-PCR, providing suitably characterized primers are available. Extracted antigens, proteins or toxins that are characteristic of species or genera may be immunoassayed, providing suitable antiserum is available. The advantages of such molecular techniques to monitor specific spores are that they can be performed on a large number of samples, often quickly and without a strong technical background in mycological techniques.

In many cases the production of suitable biomarkers by the affected host is used to detect previous exposures of pathological importance. The most common biomarkers are human IgG (of different subclasses) and IgE. The former can be used both as a function of airborne exposure and is used as a component of diagnosis of a range of fungal diseases (e.g., hypersensitivity pneumonitis). Immune responses to specific *A. fumigatus* antigens can be used diagnostically (e.g., for allergic bronchopulmonary aspergillosis in cystic fibrosis), although in this case the antibodies are the consequence of colonization of the airways, not aerosol exposure. Fungal-specific IgE is the hallmark of allergic sensitization and in some communities such allergy is the strongest risk factor for asthma [7]. Fungal allergy is the only aero-allergy identified as a risk factor for near or fatal asthma.

Our group has developed the Halogen assay, which enables the co-visualization of individual spores together with their expressed allergens, which are immunostained with IgE or IgG [8]. Thus, the method enables an environmental sample to be directly

analysed to determine which type of spores a patient is allergic to. Biotrophs (which cannot be cultured, and therefore extracts cannot be prepared for conventional allergy diagnosis) have also been identified as allergen sources by this method. Further, dual staining of *Alternaria* and *Aspergillus* spore-allergens with both human IgE and species-specific monoclonal antibodies is possible, which enables the identification of spores that are the source of allergens in situations where morphological criteria are not sufficient for their identification [in preparation]. The Halogen method also powerfully demonstrates the importance of germination in achieving allergen expression [8]. We have speculated that intra-nasal germination, rather than inhalation of numbers of spores *per se*, may form a critical event in providing 'exposure' to fungi.

The wide diversity of fungi present in environmental samples and their possession of common pathogenic components, such as cell wall extracellular polysaccharides, has led to the use of general proxies for their biomass as markers of overall fungal exposure. These markers include ergosterol and beta (1→3)-glucans [9]. While such markers may lack the ability to discriminate between species, they do integrate the diversity of exposure to a variety of viable and non-viable components, the pathogenic role of these as inducers of innate mechanisms, and the differences in biomass between spores of different species – which is about 200 fold between *Alternaria* and *Aspergillus* spores, for example.

Interpretation

Interpretation of sampling data continues to provide a challenge, particularly in a litigious society, balancing health risks with enterprises. Risk depends on the context – what may be acceptable for a dwelling or occupational setting may be a serious risk for immunocompromised patients. Concern about the health effects of domestic airborne exposure to specific fungal mycotoxins is not widely substantiated, although fungal contamination provides a significant impact on both the structural integrity and aesthetics of buildings and may affect the health of occupants. There are currently no defined exposure thresholds, although a figure of 500 spores/m³, combined with higher exposure indoors than outdoors, would appear to provide a simple benchmark for indoor sites [2]. A recent meta-analysis for asthma risk linked disease activity to indices of fungal exposure [10]. This is substantiated by studies showing increases in asthma symptoms and airways hyper-responsiveness with airborne spore levels [11], and experimental challenge studies that have

produced symptoms with levels of spores that occur in high-exposure dwellings [12].

Conclusion

After more than a century where the basic collection and analytical techniques have changed little, it is likely that the need to rapidly sample and detect biowarfare bacterial spores and toxins will contribute new methods to measure fungal exposure. Such methods range from rapid and miniature assay systems using specific antibodies, protein fingerprints or DNA that can be applied to collected samples, to spectral UV or IR backscatter systems that might detect airborne organisms at a distance. The technical challenge remains formidable – characteristic macromolecules can be difficult to extract and some are only expressed under specific conditions, while the organisms of interest occur among a rich and variable background mixture of other materials that can obscure detection. However, the legacy of this will be more rapid and accurate systems to identify both the fungal organisms and their molecules involved in human disease.

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