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Assessment of the atmospheric fungal prevalence through field ergosterol measurement I—Determination of the specific ergosterol content in common ambient fungal spores and yeast cells

Jessica Y.W. Cheng [a], Arhur P.S. Lau [b,*], Ming Fang [c]

[a] Environmental Engineering Program, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China
[b] Institute for the Environment, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China
[c] Department of Chemical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

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ABSTRACT

This and a companion paper report a means to assess fungal prevalence in ambient air by measuring the ergosterol concentration. The results are highly reproducible. Twelve species and strains of fungal spores and yeast cells commonly found in ambient air in the subtropics were cultured in the laboratory. Gas chromatography–mass spectrometry (GC–MS) was used to determine their ergosterol content. (1) The specific ergosterol content of the 12 species ranged from 0.047 to 9.919 pg (spore or yeast cell)$^{-1}$. (2) It was found that the larger the surface area of the spore/yeast cell, the higher the specific ergosterol content. (3) The specific ergosterol content of the spores or cells increased during vegetative growth of the parent cells, but became steady once the parent had matured. (4) Ergosterol was found to decompose in autoclaved spores or yeast cells, but a substantial amount of the ergosterol remained. Therefore, ergosterol can be used to reflect both the viable and the non-viable fungal mass. In a companion paper, an empirical general conversion factor for determining fungal concentration from field ergosterol measurements is proposed.

1. Introduction

Fungi are ubiquitous in air and they are known to play an important role in inducing various health problems (Kurup et al., 2002; Douwes et al., 2003; Fischer and Dott, 2003). Unlike other air pollutants, there is currently no threshold limit for airborne fungi due to the lack of comprehensive health-based data on fungal exposure, as well as the lack of an agreed protocol for measuring airborne fungi (Burge and Otten, 1999). To assess the amount of airborne fungi, culture-based analysis is most commonly used. However, the reproducibility is poor and normally only a small fraction of the fungi collected (0.3–55%) can be cultured due to sampling stress and the selectivity of culture media (Lappalainen et al., 1996; Lee et al., 2006). Alternatively, fungal spores can be enumerated directly by microscopic examination based on morphological recognition. Experienced personnel can quantify both the culturable and the non-culturable fungi by this technique, but the results are often subject to human bias. Hence, there is a demand for a quantitative and objective method for fungal assessment. The biomarker ergosterol has been proposed as a basis for such a method (Miller and Young, 1997; Robine et al., 2005; Lau et al., 2006).

Ergosterol is a primary sterol in all fungal cell membranes, but it is generally absent from most plants, bacteria and other microorganisms. Though some algae (Otsuka, 1963), cyanobacteria (Řezanka et al., 2003) and protozoa (Quiñones, 2004) do contain ergosterol in their

* Corresponding author. Tel.: +852 2358 6915; fax: +852 2358 1582.
E-mail address: pslau@ust.hk (A.P.S. Lau).

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membranes, it is in very minute amounts. Thus, ergosterol has been used to assess fungal prevalence in different environments such as soils (Bååth and Anderson, 2003), house dust (Axelsson et al., 1995), aquatic systems (Gessner and Chauvet, 1993) and bio-contaminated building materials (Reeslev et al., 2003). Although ergosterol can be precisely quantified, it cannot directly indicate the amount or mass of fungi due to inter- and intra-specific variations. To circumvent this, empirical conversion factors relating ergosterol concentrations measured in the field to fungal biomass in different habitats or environments have been suggested (Gessner and Chauvet, 1993; Reeslev et al., 2003; Klamer and Bååth, 2004). Due to natural variations such as inter- and intra-specific differences, the effect of growth conditions and the effects of nutrient sources, applying conversion factors formulated in the laboratory to field samples have usually resulted in unrealistic estimates of fungal biomass (Gessner and Newell, 2002). Gessner and Chauvet (1993) established species-specific conversion factors under near-natural growth conditions and took into account the relative abundance of the dominant fungal species to improve the estimation of fungal biomass in aquatic systems. Similar conversion factors for common airborne fungal species are essential before ergosterol concentration can be employed as a parameter to assess ambient fungal prevalence in the air.

In ambient air, spores of mycelial fungi and non-mycelial yeast cells are the prevailing structures. Although hyphal fragments may exist, they are less stable and more easily decompose or disintegrate than the intact spores or yeast cells. In this study, the term "specific ergosterol content" was adopted to describe the ergosterol content in a spore or yeast cell of a purified fungal species or strain.

To date, only one comprehensive study of the specific ergosterol content of indoor airborne fungal spores has been reported. Miller and Young (1997) measured the specific ergosterol content of dried spores of 11 fungal species commonly found in indoor air in Canada, and reported values which ranged from 1.71 to 5.11 pg spore^{-1}. They further proposed a conversion factor of 2.59 pg spore^{-1} by simply averaging the specific ergosterol content of the 11 fungal species studied. Using this conversion factor, they estimated ambient spore concentrations from the ergosterol concentrations measured in different indoor environments. Robine et al. (2005) found these specific ergosterol contents applicable to their study of airborne fungi in France. However, when Lau et al. (2006) applied this conversion factor to atmospheric ergosterol concentrations measured in Hong Kong (a subtropical environment), the estimated spore concentrations were far below the measured concentrations of viable fungi. This indicates that the suggested conversion factor may not be universally applicable, and may even be species- and site-specific. It also demonstrates a lack of understanding underlying the use of conversion factors for fungal prevalence estimation. A better foundation is essential if ergosterol is to be employed as an indicator in fungal prevalence studies.

The aim of this study was to derive a general empirical conversion factor for the calculation of fungal prevalence from field measured ambient ergosterol concentrations. This paper reports laboratory studies of the specific ergosterol contents of common airborne fungal species isolated from ambient air. Effects such as parental colonial age, viability and cold storage temperature and time that can affect the ergosterol content of spores or yeast cells were also investigated. These results provide a rigorous database for deriving a general empirical conversion factor. The derivation of the factor is reported in a companion paper (Cheng et al., 2008).

2. Materials and methods

2.1. Preparation of spore and yeast cell suspensions

The fungal species and strains used in this study were purified isolates collected in the course of long-term sampling of Hong Kong's atmosphere (Lau et al., 2002). Fungal colonies collected from the atmosphere using viable samplers (Andersen N6 single stage or six-stage samplers) loaded with malt extract agar (MEA) plates were isolated and purified. The purified fungal isolates were identified to the genus level based on the morphological characteristics of the fungal hyphae, sporangia and spores under an optical microscope (BH2, Olympus) using the identification guides (Larone, 2002; Hoog et al., 2000). The purified isolates were kept as stock cultures on MEA plates stored at 4 °C.

The stock cultures were reactivated by sub-culturing the isolates on MEA plates at 28 °C for 4 to 5 days twice before using them for the study. The reactivated cultures were aseptically transferred with an inoculating needle to fresh MEA plates, and then incubated at 28 °C for 4–23 days to study the effects to be described below. The spores were collected by gently sliding a sterilized blade over the fungal colonies and transferred to sterilized deionized water. The harvested spore suspensions were sonicated for 30 min to minimize aggregation of the spores. Yeast cell suspensions were prepared by gently shaking cultured plates filled with sterilized deionized water to loosen the yeast colonies.

The concentration of spores or cells in each suspension was determined by counting using a hemacytometer under an optical microscope (BH2, Olympus). Total spore number was counted in the two 0.1-mm^3 counting chambers of the hemacytometer for each preparation. If the difference of count between the two chambers was larger than 10%, the data were discarded. A new preparation was counted until the difference fell within 10%. Depending on the sizes of the spores or yeast cells, about 100–1000 spores or cells were counted for each preparation. The sizes of the spores or cells in terms of the length of the major and minor axes of 20–25 individual spores or cells were also measured at the same time under the microscope, which had an objective micrometer with a unit scale of 2.5 μm at a magnification of 400.

2.2. Measuring ergosterol content

Ergosterol content was determined by modifying the analytical procedures described by other researchers for