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Detection of volatile metabolites of moulds isolated from a contaminated library

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ABSTRACT

The principal fungal species isolated from a contaminated library environment were tested for their microbial volatile organic compound (MVOC) production ability. *Aspergillus creber*, *A. penicillioides*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. halophilicum*, *Penicillium brevicompactum* and *P. chrysogenum* were cultivated on suitable culture media inside sample bottles specifically designed and created for direct MVOC injection to a GC–MS instrument. The fungal emissions were monitored over several weeks to detect changes with the aging of the colonies, monitored also by respirometric tests. A total of 55 different MVOCs were detected and isopropyl alcohol, 3-methyl-1-butanol and 2-butanone were the principal compounds in common between the selected fungal species. Moreover, 2,4-dimethylheptane, 1,4-pentadiene, styrene, ethanol, 2-methyl-1-butanol, acetone, furan and 2-methylfuran were the most detected compounds. For the first time, the MVOC production for particular fungal species was detected. The species *A. creber*, which belongs to the recently revised group *Aspergillus* section *Versicolores*, was characterized by the production of ethanol, furan and 1,4-pentadiene. For the xerophilic fungus *E. halophilicum*, specific production of acetone, 2-butanone and 1,4-pentadiene was detected, supported also by respirometric data. The results demonstrated the potential use of this method for the detection of fungal contamination phenomena inside Cultural Heritage's preservation environments.

1. Introduction

The study of microbial volatile organic compounds (MVOCs) produced by moulds developed in indoor environments has been addressed by several authors, especially in relation with dampness situations and possible human health problems (Cabral, 2010; Moularat et al., 2008; Polizzi et al., 2009; Wady et al., 2003). Since the 1990s, MVOCs are often discussed to be associated with the sick building syndrome (SBS) because of their suspected role as responsible for a wide range of specific and non-specific symptoms and discomfort (Elke et al., 1999; Larsen et al., 1998; Matysik et al., 2008; Meyer et al., 1998; Wessén and Schoeps, 1996).

In the recent years, MVOCs started to be considered also for cases concerned the preservation of Cultural Heritage, as biodeterioration phenomena interested archive and library collections (Joblin et al., 2010; Pinzari et al., 2004). Fungal contamination became a frequent and complex problem to manage, often with severe, economic and health implications (Montanari et al., 2012). However, the majority of previous studies were mainly focused on the detection of chemical markers specifically related to the natural degradation of the book and paper components (Fenech et al., 2010; Lattuati-Derieux et al., 2004; Strlič et al., 2009, 2010).

The preservation of art collections starts from the performed prevention features, and MVOCs could serve as general early indicators of potential biocontamination problems (Pinzari et al., 2004). In fact, these compounds originate from both fungal primary and secondary metabolite production, strictly depending on the fungal species, the substrate and in according to the different fungal growing phases (Korpi et al., 2009; Matysik et al., 2008; Polizzi et al., 2009). Several alcohols, e.g. 1-octen-3-ol and 3-octanol, as well as ketones and furans were addressed as indicators of mould, recognised both on pure culture studied, on agar substrate, and on wallpaper and building materials (Polizzi et al., 2012). *Aspergillus* and *Penicillium* are the most investigated fungal genera in MVOC studies (Fiedler et al., 2001; Matysik et al., 2008, 2009;

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Table 1
List of fungal species isolated from the contaminated repository and selected for MVOC analysis. MUT and GenBank® accession numbers are included.

Fungal species	MUT no.	GenBank® accession number	Source
Aspergillus creber Juriević, S.W. Peterson & B.W. Horn	MUT 470	KU179486	Book cover
Aspergillus penicillioides Spegazzini	MUT 481	KU179489	Book cover
Cladosporium cladosporioides (Fresen) G.A. de Vries	MUT 527	KU179495	Indoor air
Eurotium chevalieri L. Mangin	MUT 472	(*)	Indoor air
Eurotium halophilicum C.M. Chr., Papav. & C.R. Benj.	MUT 482	KM502179	Book cover
Penicillium brevicompactum Dierckx	MUT 536	KM502183	Book cover
Penicillium chrysogenum Thom	MUT 5493	KM502200	Indoor air

(*) Fungal strain without deposited sequence in GenBank® because of its low quality.

Moularat et al., 2008; Polizzi et al., 2012; Schuchardt and Kruse, 2009; Wady et al., 2003, Wady and Larson, 2005) because of their ubiquity in indoor environments (Cabral, 2010; Samson et al., 2004), also in association with the biodeterioration of Cultural Heritage (Micheluz et al., 2015; Sterflinger, 2010; Zyska, 1997). However, in the recent years, specific fungal contamination emerged inside Italian archives and library, mainly caused by a xerophilic fungus with a lack of knowledge about its MVOC emission capability, i.e. *E. halophilicum* (Micheluz et al., 2015; Montanari et al., 2012; Pinzari and Montanari, 2011).

The determination of MVOCs is usually accomplished by gas chromatography-mass spectrometry (GC–MS) because of its powerful separation capability and highly sensitive detection performance (Matysik et al., 2009). Different sampling methods have been commonly used for sampling volatiles, as headspace solid-phase microextraction (HP-SPME) and Tenax desorption tubes, Tenax® TA, because of their low-costs and as rapid tool to determine very low quantities of a wide range of analytes (Fiedler et al., 2001; Matysik et al., 2008; Schuchardt and Kruse, 2009). Recently, other passive devices were developed specifically for long-term study requirements, as adsorbents based on activated charcoal (Matysik et al., 2009) or PDMS strips (Gibson et al., 2012). Other kind of technique is based on the MVOCs detection by sensor-based devices, e.g. polymer sensors responsive to variation in electrical conductivity due to VOC adsorption (Joblin et al., 2010) or electron noses (e-noses), based on rapid detection and identification of a preselected range of volatile compounds (Kuske et al., 2005; Pinzari et al., 2004).

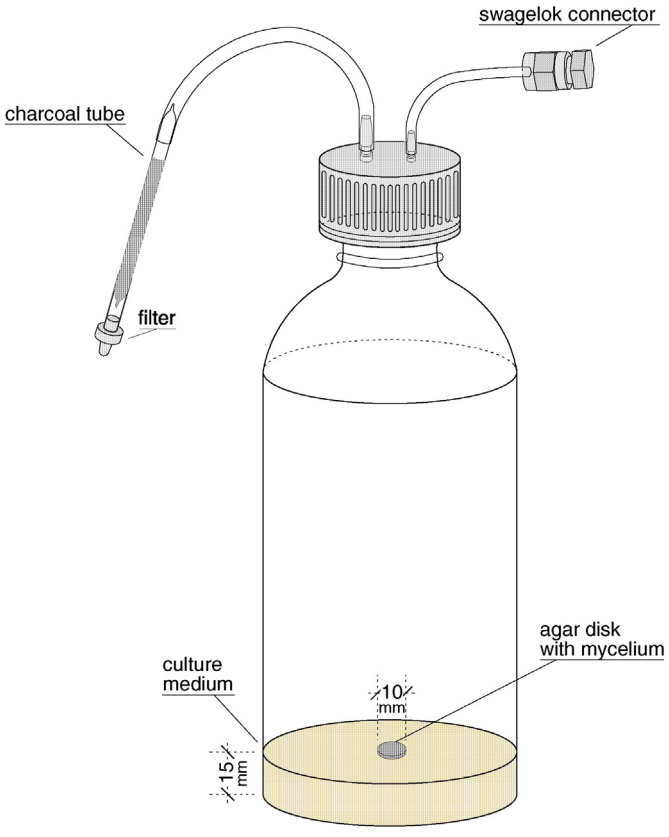

Fig. 1. Sample bottle for MVOC analysis of fungal culture.

Most of the sampling techniques require a support device for the volatile compound captures, a desorption system and, often, a preselection of detectable compounds. In order to avoid these steps and to permit the analysis of the total air composition, with the specific aim to prevent chemical artefact formation, an alternative air sampling system was presented. Based on EPA TO-15 (1999), *ad-hoc* fungal culture bottles were developed for the analysis of several fungal species isolated from a contaminated library by GC–MS. As a result of a previous work (Micheluz et al., 2015), *Aspergillus creber*, *A. penicillioides*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. halophilicum*, *Penicillium brevicompactum* and *P. chrysogenum* were tested for their MVOC production. The aim of this work was to highlight specific chemical compounds for each fungal species, comparing the results with data available in literature and improving the knowledge for the species, which never have been screened before for their volatile compound production.

2. Materials and methods

2.1. Study site

The study was focused on a repository of the Library of Humanities (Biblioteca di Area UManistica, BAUM), at Ca' Foscari University, Palazzo Malcanton Marcorà, in Venice (Italy), in which a spreading fungi contamination interested >27,000 books belonging to the Historical collections. Located in the second underground floor, the repository covers an area of about 150 m^2 and is furnished with 50 Compactus® shelves.

2.2. Sampling and fungal identification

The mycological sampling was performed for the detection of the viable airborne fungal load and for the isolation of fungi grown on books. Five sampling areas were chosen inside and outside of the repository, as reported in the previous study by Micheluz et al. (2015). Air sampling was performed in active mode by Sampl'air Lite sampler (Biomérieux, Florence, Italy) in three replicates with 9-cm Petri dishes containing different media (Malt Extract Agar, MEA and Malt Extract Agar added with 15% of NaCl, MEA15%, purchased at Fluka, Sigma-Aldrich), flow rate 100 L min^{-1} and sample volume of 100 L. Contaminated books were sampled by sterile cotton swabs (Cultiplast, PL ItalianaSpa, Milan, Italy) wiped on book covers and then inoculated onto 9-cm Petri dishes. Fungal identifications were based on macroscopic and microscopic features and confirmed by molecular techniques (Micheluz et al., 2015, 2016). All the fungal