art'14
11 th International Conference
on non-destructive investigations and microanalysis for the diagnostics and conservation of cultural and environmental heritage
June 11-13 2014 Museo Arquelógico Nacional

IND 46

FUNGAL CONTAMINATION SPECIFICALLY RELATED TO THE USE OF COMPACTUS SHELVINGS: THE CASE STUDY OF A VENETIAN LIBRARY

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Abstract

Since the last decade, several authors described a particular fungal infection associated to books in different Italian archives and libraries. The fungus *Eurotium halophilicum* C.M. Chr. Papav. & C.R. Benj (anamorph: *Aspergillus halophilicus*), a particular xerophilic fungus, which is able to grow inside climate controlled indoor environments (18-20 °C, 50-60% relative humidity), such as libraries, but also within air-stagnation areas, was pointed as the main responsible of the infections.

The same phenomenon was discovered in a library at the Ca' Foscari University of Venice. Stored in Compactus® shelves, more than 27.000 books resulted affected by a fungal infection, consisting of widespread, scattered, white spots of mycelium. The most involved parts were the exposed bindings made of leather, cotton fibers, but also coated paper.

Different sampling methods, like sterile swabs and specific transparent adhesive tapes (Fungi-Tape™), were used to sample and isolate the mycelium from the infected books, and investigate if the origin of the infection could actually be attributed to the fungus *E.*

halophilicum. Moreover, aerobiological analyses, performed by using different selective media, were carried out to characterize the book deposit environment, in order to find a possible peculiar distribution of microbial consortia and the relationship between airborne fungi and the infections observed on the material.
Results confirmed the occurrence of *Eurotium halophilicum*'s infection in the Venetian library, both on the books and in the indoor air. Moreover, from the aerobiological analysis, some other xerophilic fungi, such as some fungal strains belonging to the recently revisiting *Aspergillus* section *Versicolores*, were observed for the first time in Venetian conservation environments.

Introduction

The proper management of indoor climatic conditions of libraries or archives is often not sufficient alone to preserve books, documents and other paper supported objects from any kind of degradation phenomena. For these environments the recommended thermo-hygrometric conditions are 18-20 °C air temperature and 50-60% relative humidity according the International Federation of Libray Association (IFLA).
In the last decade, a monospecific fungal infection was discovered inside several Italian archives and libraries, especially those that adopted Compactus® shelving storage system (1, 2). These kinds of shelves are optimal for preserving the books from light degradation and dust deposits, but without an efficient climate control system, they are able to create a suitable microenvironment for specific micro-fungal growth. The fungus responsible of the infections is *Eurotium halophilicum* C.M. Chr. Papav. & C.R. Benj. (anamorph: *Aspergillus halophilicus*) a xerophilic fungus previously isolated from dry food or indoor dust, frequently in association with *Aspergillus penicillioides* Spegazzini and dust mites and, recently, from books (3, 4, 5).
In this work, a Venetian case of books' infection by *Eurotium halophilicum* and a first aerobiological survey of a library are reported.

Materials and methods

The case study was at a Ca' Foscari University's library sited in Venice (Italy), in particular a deposit located in the subbasement and characterized by the presence of only Compactus® shelves. More than 27,000 books from XVI to XIX century belonging to the Ca' Foscari historical collection were found covered by a particular white scattered fungal growth, especially those characterised by bindings made of leather, cotton fabric and coated paper (Figure 1).

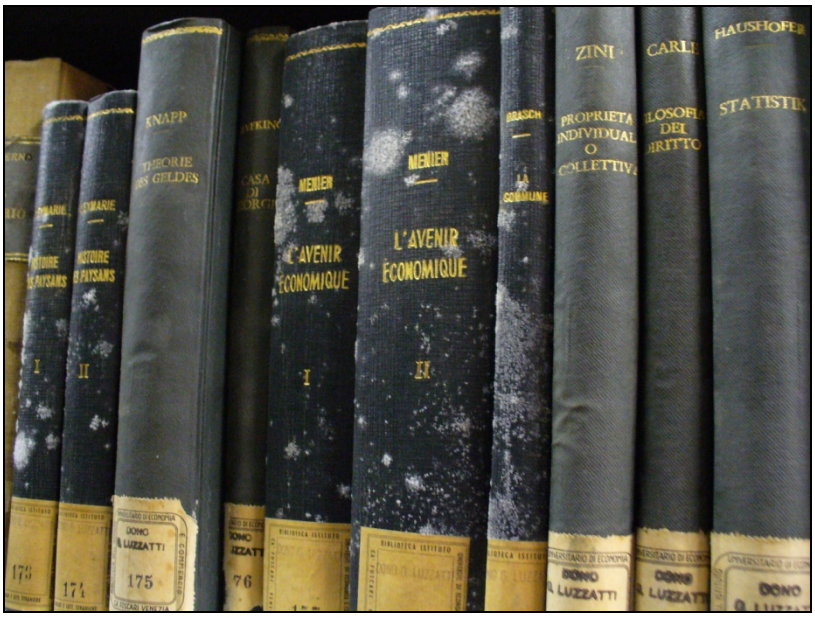

Figure 1. White fungal colonies on book's covers.

In order to characterize the infected books and environmental microbial conditions, we carried out:

- sampling of five infected books by sterile cotton swabs, wiped across fungal spots and subsequently inoculated inside Petri dishes (90 mm of diameter);
- pieces (6 × 2 cm) of Fungi-Tape™ were pressed over spots to collect fungal structures and then deposited on sterile glass slides for direct observations with Optical Microscope Axio Plan at 200x and 400x magnification;
- aerobiological sampling in five sampling areas chosen inside and outside the deposit in respect to the unique point of entrance/exit, *i.e.* in respect to the unique point of air exchange. Active sampling was performed with a Sampl'Air Lite sampler with 90 mm Petri dishes, a flow rate of 100 L/min and a sample volume of 100 L. The sampler was placed 1.5 m above the floor to represent the breathing zone of a steading person and the results are presented as *colony-forming unit per cubic meter* (CFU/m^3).

To verify the presence of general xerophilic and halophilic microorganisms, and in particular of the *Eurotium halophilicum*, Malt Extract Agar added with 150 g/L of NaCl (MEA 15%) supplemented with chloramphenicol was used. All Petri dishes were incubated at 25 °C for 7-14 days (3, 4).

After the incubation period, each representative fungal colony was isolated through disposable sterile loops and transferred on Malta Extract Agar (MEA) and MEA 15% for 7 days of incubations at 25 °C. The fungal colonies were identified to genus level by observing their macro- and micro-morphological features (6, 7, 8, 9). The species identifications were carried out coupling molecular and morphological analyses.

Results

Direct observations of Fungi-Tape™ samples by the optical microscope showed the numerous presence of different fungal structures, as mycelium, conidiophores and conidia, most of them belonging to *Aspergillus* spp. In particular, uniseriate radiate conidiophore (Figure 2) heads were recognised and different sizes of conidia were collected, 5-7.5 × 5-9 µm, probably conidia and ascospores belonging to both *Aspergillus* spp. and *Eurotium* spp.

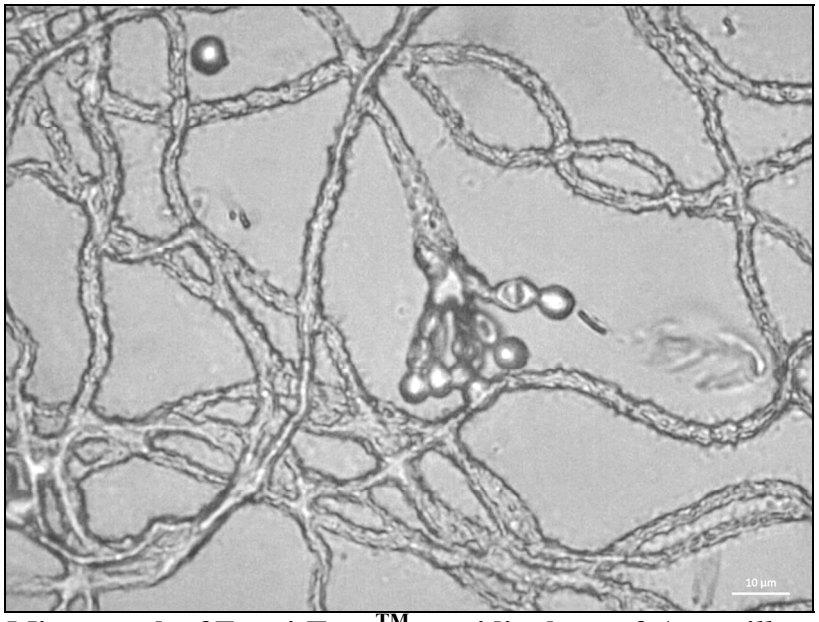

Figure 2. Micrograph of Fungi-Tape™: conidiophore of *Aspergillus* sp., 400X.

From book samples, white to cream colonies slightly depressed in the middle were slowly grown on MEA 15%. By optical microscopic observation and confirmed by molecular analysis, typical spiral-shape ascoma initials of *Eurotium halophilicum* were recognized (Figure 3), together with green colonies of another xerophilic fungus, *Aspergillus penicillioides* Spegazzini. Already in 1978, these fungi were reported together by Samson & Lustgraaf as cohabiting in house dust, emphasizing the possibility to be responsible of allergic reactions and lung disease (4).

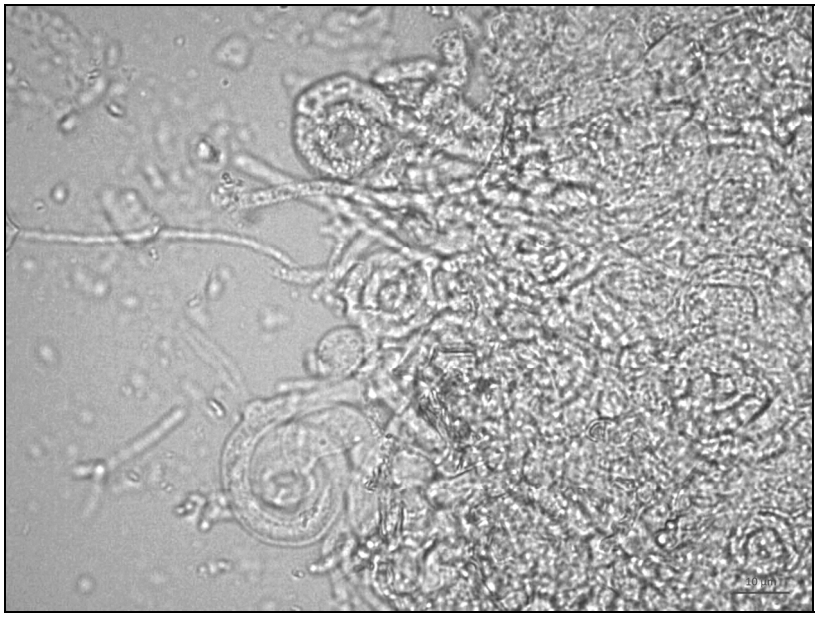

Figure 3. Micrograph of *E. halophilicum* colony: particular of spiral-shape ascoma initials, 400X.

The aerobiological sampling showed a high variability, as reported in Table 1. Different values of airborne contamination and numbers of fungal taxa were observed inside the book's deposit. Areas 1 and 2 were the most infected areas with average values (standard deviation in brackets, STD) of 2000 (±610) CFU/m^3 and 1620 (±80) CFU/m^3, respectively. These values were high in comparison with those obtained from the other sampled areas which showed a range of contamination values between 29 (±13) and 101 (±12) CFU/m^3, but also respect to the thresholds recommended by regulations (150 CFU/m^3) (10).

Table 1. Fungal air contamination values in the librarian deposit.

Sampling areas	Average active sampling and STD (CFU/m^3)	Number of fungal taxa
1: Right corner far from the entrance, between shelves	2000±610	6
2: Corridor close to the area 1	1620±80	8
3: Left corner far from the entrance, between shelves	81±1	17
4: Entrance	101±12	26
5: Storage room adjacent to the deposit studied	29±13	8

The morphological results and the molecular characterisation of the aerobiological sampling highlighted the presence of different fungal patterns in the various sampled areas. About 30 different fungal species were identified, most of which belonging to the typical indoor fungal genera *Aspergillus* spp. and *Penicillium* spp. (11).
The transition areas were characterized by a higher fungal diversity (26 different taxa), possibly because of workers and students presence and book's handling. On the contrary, from the areas 1 and 2, a limited number of fungal species were isolated (6 and 8 taxa), which might be due to the conditions of the close shelves, like low air exchange rates compared to the transition areas.
In addition to *Eurotium halophilicum*, the species isolated with high frequency was *Aspergillus creber* Jurievic S. W. Peterson & B.W. Horn. This fungus and the other detected species, *i.e. Aspergillus jensenii* Jurievic S. W. Peterson & B.W. Horn and *Aspergillus protuberus* Muntañola-Cvetković, all belonging to the recently revised group *Aspergillus* section *Versicolores* (12). This is the first observation of these fungi in a Italian library environment.

Conclusions

A fungal infection caused by a proliferation of *Eurotium halophilicum* has been detected inside a Ca' Foscari Library. The presence of this fungus has been recorded from both books and in the surrounding air, often in association with the fungus *Aspergillus penicillioides*, probably because of their similar ecological niche and low water requirements. As previous reported infections from different Italian archives and libraries, the books were stored inside Compactus® shelves remained closed for a long time without a sufficient air-exchange. As suggest by many studies, this species was underestimated

because of its particular cultivation requirements, but probably its presence is widespread in Italian deposits.
From the sampled air, *Aspergillus creber*, *Aspergillus jensenii* and *Aspergillus protuberus* belonging to the *Aspergillus* section *Versicolores* group were detected for the first time inside an Italian library environment.

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