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Cultural Heritage Environments: Monitoring Strategy for Preventive Conservation of Cultural Assets and Human Health Protection

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Abstract:

Objects of historic artistic value, conserved in indoor deposits or exhibited inside museum halls, are strongly influenced by the environmental parameters, as temperature, relative humidity and light quality. Environmental parameters directly impact the structural integrity of constitutive materials and promote microbial colonization on artwork surfaces, leading to biodeterioration. In cultural heritage dedicated environments (CHE), the microbial load may exist both on art works surface and in the environmental aerosol (bioaerosol), maintaining a unique balance.

In this study, through a multi-phasic approach the presence of bacteria and fungal colonies in the aerosol and artifacts surface, of an exposure hall, have been investigated. This study defined specific, non-invasive procedures to sample microbial colonies, spread both on artworks surface and in the aerosol of dedicated indoor environments.

Results from morphological analysis (microscopy, *in vitro* culture) and molecular investigation (microbial genomic DNA), provided useful information on the composition of the microbial consortia, allowing a complete understanding. Microorganisms, in addition to inducing artifacts biodeterioration are able to produce and release, in the aerosol (bioaerosol) of surrounding environment, biological particles and molecules (spores, cellular debris, toxins and allergens), potentially dangerous for the health of operators and visitors.

The complete understanding of the consortia is peculiar to counteract the microbial colonization, also performing green strategies.

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1. INTRODUCTION

The biodeterioration of works of art is a complex process correlate to a wide number of microbial species, mainly fungi and bacteria, that can be concentrated in confined environments such as archives, libraries, underground environments, museums. Their growth and diffusion are closely related to physical-chemical peculiarity of the substrates and to the environmental parameters (temperature, humidity, light), considering the indoor air as a dynamic system, in which biological particles can be transported and shifted from one site to other [1-4]. Some bacterial and fungal species are able to release the products of their metabolic activities on both artefacts surface and in the surrounding environment, inducing the biodeterioration of the artefacts, and potentially becoming dangerous for the health of operators/users, releasing cellular fragments, toxins and bioactive molecules in the aerosol of exhibition/conservation environments [5-8].

Cultural heritage environments (CHE), such as museums and archaeological sites, require continuous monitoring to preserve artifacts and protect human health. The issues related to CHE safety are very complex, but they represent an essential aspect in the management of all interventions [9-11]. The study outlines a comprehensive monitoring strategy focused on preventive conservation and health protection within cultural heritage settings aimed at preserving the cultural assets and human health.

Confined environments are characterized by peculiar exchanges with the external environment, can present several problems related to the structural characteristics, the geographical position, location and intended use, taking in account the wide range of potential risks for healthcare.

A targeted diagnosis relating to environmental biological contamination represents the fundamental premise for the assessment and management of biological risk.

The development of an interdisciplinary protocol, which combines microscopy analysis, *in vitro* cultures and molecular investigation, for the overall assessment of microbial contamination of air and surfaces in confined environments, allows the definition of the "Indices of Attention or Risk" [12]. "Attention index", correspond to the presence of microbial taxa do not able to induce immediate biodeterioration (the procedure of monitoring must be repeated in a scheduled manner, preferably each 6-12 months), in order to detect changes in the taxa that constitute microbial consortia. Instead, "Risk index", agree to the presence of microbial taxa that are able to quickly induce deterioration process, accelerating structural change in the constitutive material. Microscopic investigation, as well as *in vitro* cultures, allowed the morphological characterization of the microbial species colonizing a CHE. In order to complete the identification of microbial taxa, at both genus and species level, biotechnologies have provided innovative, rapid and specific procedures in recent years [13-15]. These are based on the analysis of the microbial genomic DNA molecules, using specific molecular biology techniques which allow for *in vitro* amplification of target sequences (PCR), sequencing and sequence analysis of PCR products. Thus, allowing us to distinguish single microbial individuals even in complex consortia.

A standard monitoring strategy for CHE, not only preserves the cultural assets for future generations but also safeguards human health; by implementing comprehensive monitoring practices, communities can foster environments that exposed/stored heritage asset thanking into account the operators and visitor well-being.

EXPERIMENTAL SETUP

Sampling

Microbial particulate on the artifacts surfaces was performed with non-invasive methods [4, 16], using fragments (3x3 cm) of sterile Nylon membranes (Amersham, Hybond N+, positive charged) or sterile swabs. The Nylon fragments were positioned on the artifacts surface for 5 minutes, instead by sterile swab a 3x3 cm squared area was sampled (Fig. **2b**), applying light pressure in both cases.

The sampling of the aerosol biological matter was performed by Airport-MD 80 (Sartorius, portable sampler), filtering 0.5 m^3 of air per sample, using sterile gelatin membranes (Sartorius, gelatin disposable membranes, 80 mm in diameter); the flow rate was 2 m^3/h, performed for 15 minutes. The high efficiency of these membranes allowed the trapping of particles up to approx. 1 μ–millimeter in diameter.

Microbial Taxa Identification

Surface samples: Nutrient or Sabouraud agar (DIFCO) Petri dishes of 9 mm in diameter, were inoculated by Nylon membrane fragments (Fig. **1a**) and sterile swabs (Fig. **1b**); all plates were incubated for 24-36 hours in

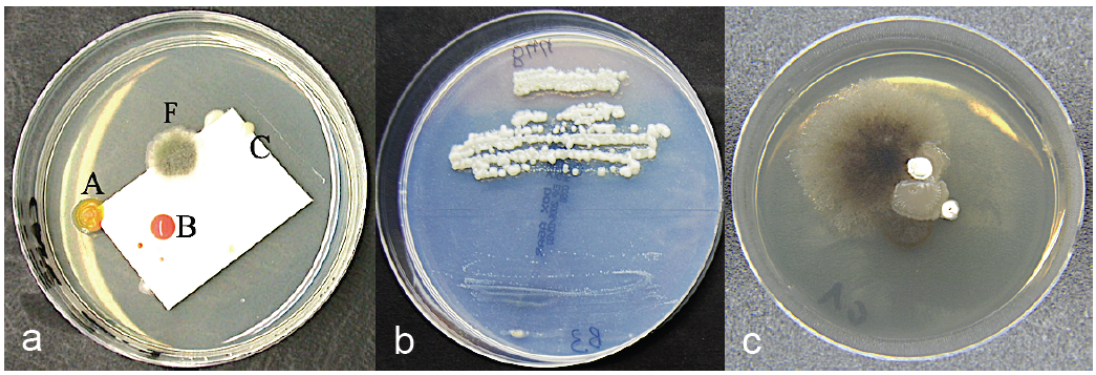

Figure 1: a) Surface sampling by Nylon membrane fragments (A, B, C = bacterial colonies; F= fungal colony); **b**) Surface sampling by sterile swab, bacterial colonies; **c**) Aerosol sampling by Sartorius Portable AirPort MD8, equipped with gelatin filters, fungal colonies are recognizable.

an oven at 30°C. Specifically, Nutrient agar (0.5% di peptone, 0.3% yeast extract, 1.5% agar, 0.5% NaCl) is a non-selective medium used for growing a wide variety of microorganisms; Sabouraud agar (40 g/L dextrose,10 g/L peptone, 20 g/L agar), containing an high concentration of dextrose and acid pH, allows the selectivity of fungi.

Aerosol samples: Based on the peculiarity that gelatin membranes are soluble in aqueous solutions, fragments were left to dissolve in the aqueous component of agar (3 minutes at room temperature), in inoculating the Nutrient or Sabouraud media for *in vitro* microbial culture (Fig. **1c**); to perform molecular investigation, filter fragments were dissolved in 0.5 ml of 10 mM Tris-HCl pH 8.0/ 0.1 mM EDTA solution (4°C for 10 minutes), carrying out the direct extraction of the microbial genomic DNA (gDNA).

The gDNA, both from isolated colonies or from aerosol collected particles, was extracted by the Gene JET Genomic DNA purification kit (Fermentas), representing the template molecules in Polymerase Chain Reaction (PCR), in order to amplify ITS - rDNA target sequences. Specifically, ITSF= 5'-GTCGTAACAAGGTAGCCGTA-3' and ITSR= 5'-GCCAAGGCATCCACC-3' were the primers for bacteria, instead the oligonucleotides ITS1= 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4= 5'-TCCTCCGCTTATTGATATGC-3' were for fungi (Fig. **2**). The nucleotide composition of obtained PCR amplicons was determined by MWG-Eurofins Service (Germany) and the sequences analysis performed by BLAST platform (EMBL-Germany, NIH-USA nucleotide databases) [17-21].

Fungal hyphae and conidia from isolated colonies, were also directly observed by optical microscope (Leica), after Lugol's iodine staining (Fig. **3**).

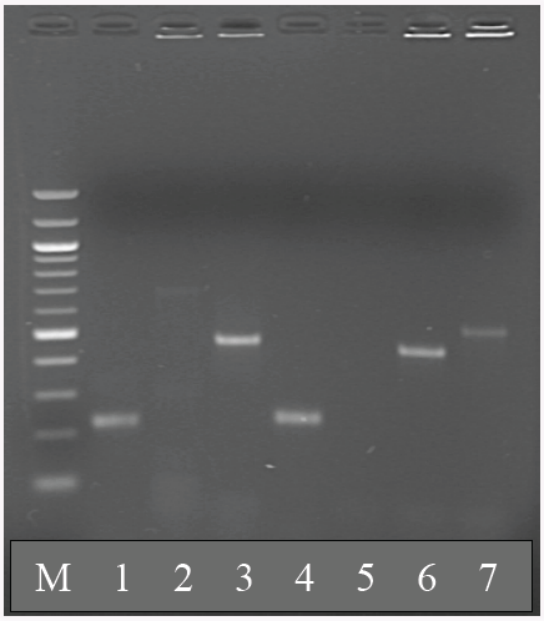

Figure 2: Agarose (1.5%) gel electrophoresis of PCR amplicons, related to bacterial (1, 2, 4) and fugal rDNA target sequences (3,6,7); M = 1kb DNA ladder (BioLabs).

Monitoring of Thermo-Hygrometric Parameters

The environmental parameters Temperature (T) /Relative Humidity (RU) in this study were continuously measured using HOBO systems, specifically T= 19+1.5°C and RU= 60+2.5%.

RESULTS

The multi-phasic approach proposed in this study, allows the identification of several bacterial and fungal colonies. Specifically, *Arthrinium* sp., *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria alternata*, *Cladosporium herbarum*, *Scopulariopsis brevicaulis* as fungi, and *Microbacterium* sp., *Streptomyces* sp., *Bacillus subtilis*, *Staphylococcus aureus* as bacteria, were mainly revealed in the aerosol samples.

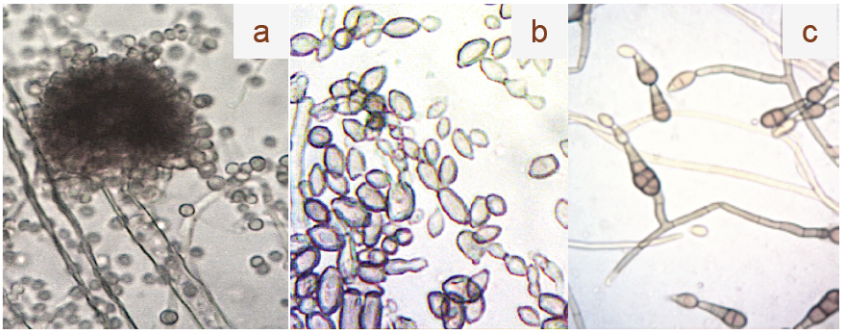

Figure 3: Fungal hyphae and conidia (*Leica* Optical Microscopy, 40X), after Lugol staining: a) *Aspergillus* sp.; b) *Cladosporium cladosporioides*; c) *Alternaria alternata*.

A quite similar microbial colonization has been revealed on artworks surface consisting of fungi as *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp., *Chaetomium globosum, Penicillium crhysogenum,* and bacteria as *Bacillus* sp*., Staphylococcus* sp., *Bacillus cereus, Bacillus simplex, Micrococcus luteus, Pseudomonas rhizosphaerae*.

The microorganisms, summarized in Table **1**, in addition to being able to colonize organic or inorganic substrates, and the air of indoor environments, are potentially pathogenic to humans or cause opportunistic infections [5, 10, 22, 23].

DISCUSSION AND CONCLUSION

Microorganisms such as bacteria and fungi are present in all habitats on earth, with a metabolic ductility that allows them to colonize substrates of different nature. In indoor dedicated environments such as museums, archives, libraries, deposits, these microorganisms developing rapidly, taking part in the formation of complex consortia colonizing the artworks surface as well as the environmental aerosol (bioaerosol). Their development is closely related to both environmental parameter (temperature, humidity, light) and physical-chemical characteristics of the substrates. Furthermore, particular attention must be paid to the products of these microbial consortia, that in addition to induce the artefacts biodeterioration can constitute a risk to the health of operators and visitors.

In this study, a multi-phasic approach was defined and applied in order to recognize the structure and taxa of microbial consortium, involved in artifact deterioration and detrimental for health of operators and visitors. The revealed microbial colonies strongly agree with those described in literature, such as bacterial belonging to *Bacillus, Micrococcus, Streptomyces, Actinomyces* and fungi as *Penicillum, Cladosporium, Aspergillus, Alternaria, Trichoderma, Rhizopus*; which in addition to the artefacts surface can be present in the aerosol of confined environments, where cultural assets are exposed/preserved.

Considering the microbial specie, potential dangerous for human health, were identified *Arthrinium* sp., *Cladosporium herbarum*, that are known as potential

Table 1: Microorganisms Revealed Onto Artworks Surface and in Aerosol of Indoor Environment

Organisms	Artworks Surface	Indoor Aerosol
Bacteria	*Bacillus* sp. *Bacillus cereus* *Bacillus simplex* *Micrococcus luteus* *Pseudomonas rhizosphaerae* *Staphylococcus sp.*	*Microbacterium* sp. *Streptomyces* sp. *Bacillus subtilis* *Staphylococcus aureus*
Fungi	*Alternaria* sp., *Cladosporium* sp. *Penicillium* sp., *Chaetomium globosum* *Penicillium crhysogenum*	*Arthrinium* sp., *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria alternata* *Cladosporium herbarum* *Scopulariopsis brevicaulis*

allergens producers; *Penicillium brevicompactum*, often present in indoor environments, producer of mycotoxins; *Scopulariopsis brevicalus*, a toxic species responsible for various infections, such as endophthalmitis, keratitis, pulmonary infections in immunocompromised subjects; *Aspergillus versicolor*, an opportunistic pathogen, agent of aspergillosis, onychomycosis, carried by dust and often present on wooden artefacts; *Chaetomium globosum*, an ascomycete that attacks wooden substrates generating "soft decay". The integrated approach showed in this paper allow to minimizing the sample amount (applying non-invasive sampling) needed for understanding the complexity of microbial communities, also revealing unculturable species.

A complete identification of microbial taxa is strictly related to biocide choose to counteract microbial colonization, also in the prospective to implementing green conservation strategies in order to replace synthetic chemical biocides [24]. Many biocides are difficult to degrade, becoming persistent in the environment, also causing contamination of the areas that are far from the site of treatment. Even though, the toxicity and persistence of these compounds are well known, as well as several factors such as toxicity to humans, risks of environmental pollution and compatibility with substrates, innovative molecules need to be found, [nowadays, collection of articles, focused on green and sustainable strategies for heritage conservation, are fully illustrate [19, 24-26].

Each cultural heritage environment has its own geographical, structural and of use characteristics, then specific microclimatic conditions, these must be constantly monitored, and carried out in combination with microbiological monitoring, as suggested in this study.

Furthermore, the monitoring of other parameters are to be considered, giving a more complete insight in environmental 'air aggressiveness', such as the particulate matter (PM) and especially its concentration, size distribution and chemical composition. Particularly, PM_{10} and $PM_{2.5}$ ultrafine particles can penetrate deep into the respiratory track, resulting in adverse human health impacts (respiratory problems, cardiovascular and respiratory mortality and morbidity, lung and brain disease) [27-30]. The insights from these datasets, combined with the traditional environmental monitoring (temperature, relative humidity and light intensity), and microbial monitoring, could allow to draft recommended guidelines for cultural heritage environments, identifying potential risks invisible when only traditional parameters are considered.

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