BIOTECHNOLOGY FOR MICROBIAL MONITORING OF INDOOR CULTURAL HERITAGE ENVIRONMENTS

DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

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An integrated approach for the characterization of the airborne particulate was employed in different sites of historical/artistic interest, with peculiar architectural structures, thermo-hygrometric and lighting parameters. In these indoor/semiconfined environments different artworks are preserved: mural paintings, stone-works, paper or parchments that are susceptible of microbial colonization. The presence of fungal spores and low air change can induce the biodegradation of manufacts, but can have potentially effect on human health (visitors/operators). Non-invasive sampling is carried out on surfaces (Nylon membrane or sterile swab), while by a portable sampler (Sartorius MD8) equipped with gelatin filters, the biological particules in the aerosol have been also sampled. Microbial consortia is revealed and characterized by Optical, Scanning Electron and Confocal Laser Scanning Microscopy (OM, SEM, CLSM), *in vitro* culture and molecular analysis (PCR, sequencing, sequence analysis). The inter-disciplinary approach applied in this study, represents a valuable contribution for define a protocol to prevent artifacts biodeterioration and to evaluate the potential health risk for visitors and operators, according to the conservative restoration procedures.

MICROBIAL SAMPLING

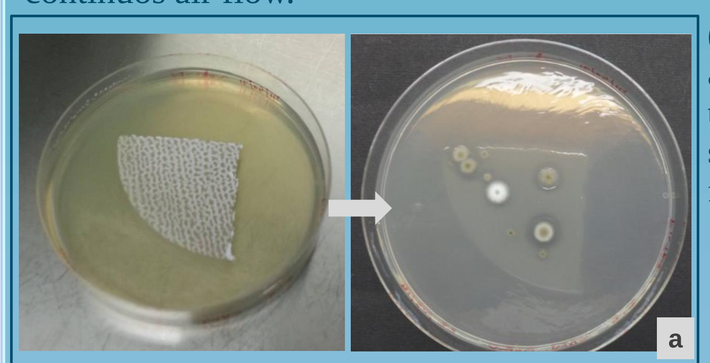
(a) Surfaces sampling by sterile swab and Nylon membrane
(b) Air Port MD8 Sartorius equipped with water soluble gelatine filters utilized for aerosol sampling

GOAL OF THE STUDY

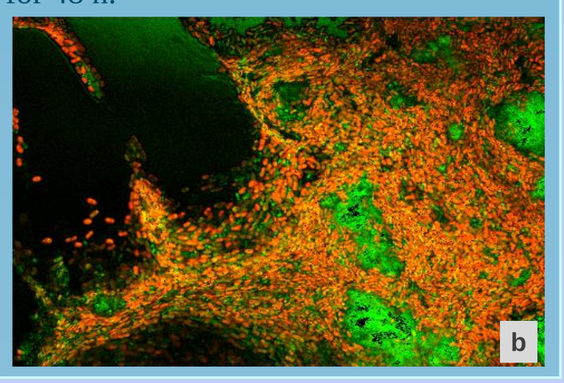
DETECTION AND CHARACTERIZATION OF MICROBIAL CONSORTIA POTENTIAL BIODETERIOGENS

Saints Cave in Licodia Eubea (Catania, Sicily)

The *Saints Cave* is a semi-confined environment (*fresco*) which is characterized by the presence of a biological airborne particulate, coming from the countryside and vehicled by a continuos air flow.



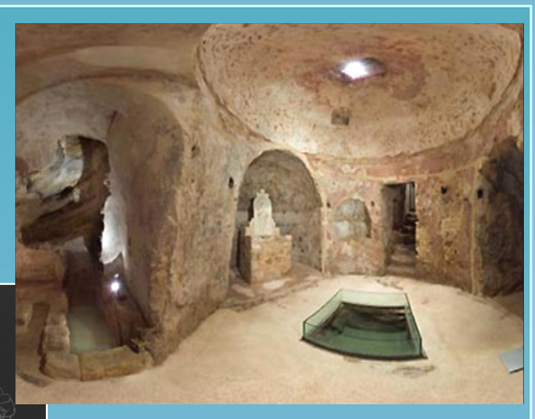
(a) Fungal colonies grew on Nutrient agar plates inoculated with fragments of the gelatine filters used for bioaerosol sampling. The plates are stored at 30° C for 48 h.

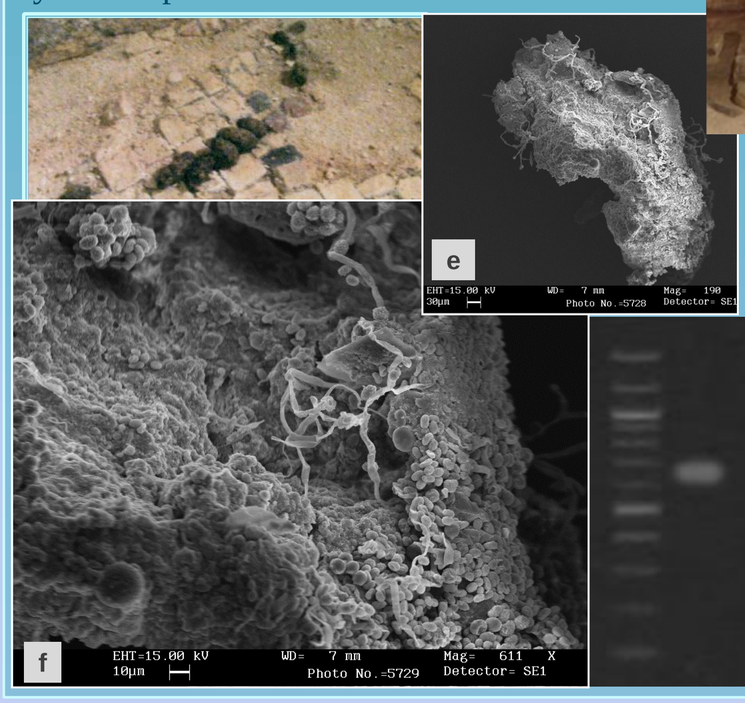


(b) CLSM micrograph (Olympus FV-300 Argon laser 488nm, green light, and elio/neon 543 nm, red light) which shows autofluorescent colonies related to the presence of microalgae and cyanobacteria.

Sibilla Antrum (Trapani, Sicily)

The *Sibilla Antrum* a hypogeal environment (*fresco*), where biological particulate can be vehicle by visitors or sometimes by synanthropic roditors.



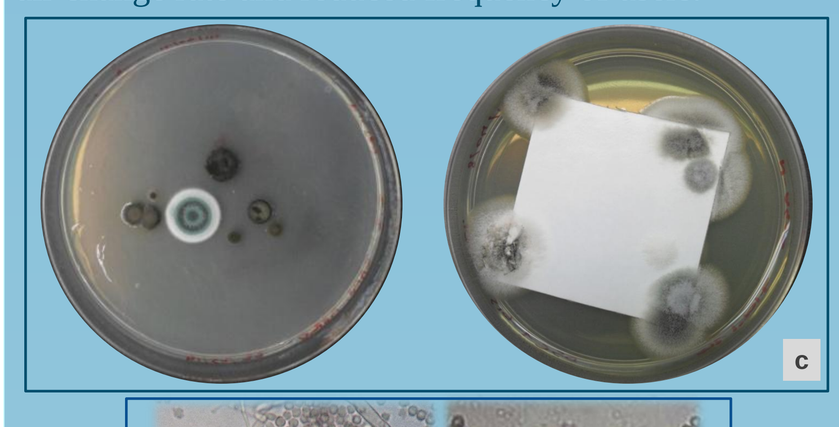


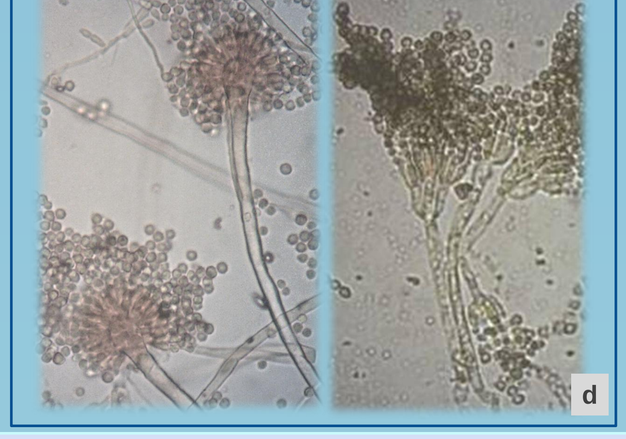
(e), (f) SEM micrographs of *Rattus* sp. droppings, colonized by microorganisms.

(g) Electrophoresis on agarose gel (2%) of the PCR products (ITS rDNA). M=100bp DNA ladder (*BioLabs*). Sequencing and comparison with genomic libraries allowed to identify, *Auxarthron* spp., one of keratinophilic fungi species.

Diocesan Historical Archive (Palermo, Sicily)

The *Diocesan Historic Archive*, an indoor-environment (documentary funds, IX-XX sec.) characterized by low air change rate and reduced frequency of users.





(c) Fungal colonies growth on agar medium inoculated by gelatine membrane and Nylon membrane fragments.

(d) OM micrographies (Lugol's solution, 40X magnitude) show the typical structures related to *Aspergillus* (left) and *Penicillium* (right) genera.

Crypt Cathedral Treasury Museum (Palermo, Sicily)

Crypt site, an underground environment (lithic and stone artifact), daily visited by tourists and characterized by a reduced indoor-outdoor exchange.

(h) *Alternaria* spp. colonies growth on agar medium inoculated by gelatine membrane fragment and reproductive propagules **(i)** stained by Lugol's solution, observed by OM (40X magnitude); **(l)** agarose gel (2%) showing the PCR-products obtained by amplification of ITS (Internal Transcribed Spacer) regions. Molecular marker 100bp DNA ladder (*BioLabs*). Sequencing and sequences homology analysis identified different microbial taxa.

CONCLUSIONS

Biodeterioration of works of art, both inorganic and organic, is a complex process involving a high number of microbial species, in particular, fungi and bacteria frequently associated with green algae, cyanobacteria and lichens. The several differences of the analyzed environments allowed to establish a common methodology for biological investigation and to characterize both microbial colonization in aerosol and widespread on artworks surfaces. Combining the results from microscopy observations, *in vitro* culture and molecular analyses we are able to describe the almost complete composition of microbial consortium. This interdisciplinary approach is essential for understanding the microbial deterioration in indoor environments, leads to the definition of the indices of "attention" and "risk" for both deterioration of cultural assets and of human health (visitors/operators).

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