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Co-occurrence of bacteria and fungi and spatial partitioning during photographic materials biodeterioration

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ABSTRACT

The microbial spoilage and correlated surface changes of a cellulose nitrate negative film, a gelatine–silver positive print, a cardboard frame and a cellulosic envelope dated back to 1938–1940 was assessed by means of molecular methods and scanning electron microscopy (SEM). Materials characterisation was obtained with Raman, Infrared and Electronic dispersion spectroscopies. DNA was extracted from bacteria and fungi, amplified through PCR oriented to bacterial 16S rRNA gene and fungal internal transcribed spacer (ITS), and clone libraries were constructed for each investigated material. The ITS fungal cloning was able to detect a bigger spectrum of species respect to bacterial one. Correspondence between molecular results and SEM observations was used to address the cause of biodeterioration to single species, and to map the presence of different organisms in separate niches. This investigation highlighted a co-occurrence of both bacteria and fungi on most of the substrata, and a spatial partitioning according to the different photographic materials. Moreover, for the first time, the effects of a biological attack on glassine paper were documented.

1. Introduction

While effects of biodeterioration (and decomposition) may be global, microbial systems interact with their environments at microscopic scales [51]. To generate hypotheses regarding the interactions within a community that result in observed patterns in diversity and richness, the relevant physical, chemical and biological factors must be measured [24]. A partitioning of the available space between spoiling species based on functional and metabolic attitudes can be hypothesised as occurring on multi-materials artefacts. A microbiological study in this direction was undertaken using as model system a "conservation unit" made of a cellulose nitrate negative film, a gelatine–silver positive print, a cardboard frame and an envelope dated back to 1938–1940, all stored together in bad conservation conditions.

Microorganisms represent dangerous agents for photographic materials and are a major cause of damage for these fragile materials of cultural value. However, few studies so far have been addressed to biological defacement of photographic collections, and even less were based on culture-independent methods. In this work the microbial diversity on photographic materials stored together at the "Archivio Ente EUR e Archivio Centrale dello Stato", Italy (Fig. 1), were assessed via DNA molecular methods and scanning electron microscopy (SEM). A previous study on the same objects, performed by means of classic culturing, and based on the use of several culturing media, provided no statistically significant results, possibly because the organisms were dead or not culturable [29].

Fungi and bacteria possess a range of important roles and functions related to organic materials degradation, which can be often associated to nutrients uptake and organisms' growth. Biodegradation of organic materials can lead to very complex biodeterioration phenomena on objects representing our cultural

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Fig. 1. The film, the envelope and the gelatin-silver prints dated back to 1938–40 and conserved at the "Archivio Ente EUR – Archivio Centrale dello Stato", Italy, analysed during the survey.

heritage [15,31,37,39]. When cultural heritage is attacked by microorganisms, the persistence and extinction of a bacterial or a fungal species colonising the objects can vary with time and environmental conditions, and strongly depends on the availability of nutrients and eventual competition with other species for their use. Moreover, the biological traits of individuals/species are not the only reasons for fungal and bacterial successions, but rather biological patterns and processes play a decisive role in the way by which species are assembled in both space and time, as this has been recognised for a long time in plant communities [39,50]. Photographic positive and negative supports are composed of at least three components: a rigid support (generally plastic or paper and sometimes glass and other materials), an image-forming material (black and white images are formed by metallic silver particles) and a binder that in 20th century documents is mainly based on gelatine. Gelatine is a mixture of high-molecular-mass polypeptides produced from collagenous animal tissues that was used for all silver halide-based photographic materials (Lourenço et Sampaio, 2009). The chemical cross-infection of different polymers stored together was recently studied [10], while the analysis of the effects of a biological attack on the different polymers contained in a "conservation unit" represents a new approach. Biological damage on films collections was studied in particular by Refs. [2,3] who isolated both bacteria and fungi from cinematographic films. Several fungal strains were associated to photographic and cinematographic films biodeterioration, mainly in the *Aspergillus* (i.e. *Aspergillus ustus*, *Aspergillus nidulans* and *Aspergillus versicolor*) and *Penicillium* genera. The chief limiting factor that determines fungal development on photographic materials is recognised to be water, although many xerophilic/halophilic fungal and actinomycetales genera were also associated to these materials. Moreover, the activity of some microorganisms can be addressed also towards some inorganic compounds present in the organic substrates [46] showed the occurrence of silver nanoparticles accumulation on the surface of fungal cell wall during biodeterioration of the silver halide-based photographic layer. One or more of the fungal strains that caused the damage were able to reduce the silver ions and to form silver nanoparticles on the cell wall, presumably capped on structural proteins [46].

The traditional methodology employed for the characterisation of bacterial and microfungal assemblages on photographic materials is direct observation based on the appearance of coloured, dusty or sporulating structures on the substrate, or pigmented stains and microscopic tracks. Identification of in vitro isolates obtained by using classic cultural techniques can allow to detecting viable but not necessarily active species [31,32], namely species that participate to the spoilage of materials, and are not casually present on them. Genomic methods are powerful but suffer from potential incomplete extraction of DNA [34], and there is a lack of diagnostic sequences in databases for many environmental bacteria and conidial fungi [42]. Moreover, DNA-based techniques alone yield no information on active species and microbial/fungal functions as biodeteriorating agents. Apart from the highly technological approaches that can be adopted in some studies for the identification of single species, the link between a peculiar damage on photographic materials and actively growing bacteria and conidial fungi in films and prints is still lacking, or otherwise poorly understood. On the basis of abovementioned indications an analysis strategy involving the combination between observation of materials by means of Variable Pressure Scanning Microscopy and molecular cloning from samples obtained from the same objects was performed. The proposed approach can in some cases disclose the real role of microorganisms in investigated materials, and their actual distribution and interaction with substrata.

2. Materials and methods

2.1. Photographic materials

The photographic materials belonging to "Archivio Ente EUR e Archivio Centrale dello Stato" were analysed by means of non invasive techniques. These "objects" can be considered as a "conservation unit" because they are typically stored together, and are therefore subject to the same conservation history and sequence of events. The conservation unit, and in particular the envelope containing the film and the print, appeared, at the naked eye, affected by moulds and other microbial stains both outside and inside (Figs. 1 and 2). All the materials were characterised by means of