Microbiological Research 207 (2018) 203–210

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Evaluation of fungal community involved in the bioderioration process of wooden artworks and canvases in Montefeltro area (Marche, Italy)

Luigia Sabatini*, Maurizio Sisti, Raffaella Campana

Department of Biomolecular Sciences, Division of Toxicological, Hygiene and Environmental Sciences, University of Urbino Carlo Bo, Urbino, Italy

ARTICLE INFO

Keywords:
Fungi
Biodeterioration
Artworks
Montefeltro area
Cultural methods

ABSTRACT

Microbiological monitoring represents one of the most useful methods to assess potential risks related to the integrity of cultural heritage. The objective of this work was to evaluate the fungal community prevalent in 64 different artworks of Montefeltro area (Marche, central Italy). A total of 293 swabs were collected and, among these, 2.3% resulted negative to cultural method, while 87.7% were positive for the presence of filamentous fungi, yeasts and Actinomycetes. Totally, 11 genera and 12 different species were recovered from Sabouraud Dextrose Agar (SDA), Czapek Dox Agar (CDA), Malt Extract Agar (MEA), including 73 strains of *Mycelia sterilia*. *Penicillium* spp. was isolated mostly from canvases (28.8%), while *Aspergillus* spp. was most present in wooden artworks (25.3%). SDA was the best performing medium with 57.1% of isolations, followed by CDA and MEA with percentages of 24.8 and 18.1% respectively.

This study could be useful to better understand the microorganism-related phenomena in cultural heritage of Marche region, identifying the potential risks and defining preventive protecting such as climate control, frequent cleaning and environmental monitoring.

1. Introduction

Biodeterioration is a relevant problem in the conservation of cultural heritages because they represent a widely diversified group of ecological niches for various organisms. There are a number of abiotic and biotic factors that have deteriorating effects on materials used for artworks, such as humidity, light, pollution, temperature, and several types of microorganisms (Jerusik, 2010; Pepe et al., 2010; Sterflinger, 2010). Among these, fungi seem to be a predominant feature in museums and warehouses of many countries all over the world (Valentin, 2010; Sterflinger and Piñar, 2013). In the indoor environment, unlike bacteria which growth is limited by their need for water, fungi are able to grow at relatively low levels of temperature and humidity, in quite harsh conditions, fungal spores are able to survive for a long time (Florian, 2002; Vukojeviç and Grbiç, 2010). Fungal growth may be recovered in the support materials (cellulose, canvas, wood, parchment, silk, and wool), in the materials used to adhere the paintings to the support (animal or plant glues), as well as in the organic binders in which pigments are emulsified (oils, waxes, polysaccharides) (Ciferri, 1999). Depending on the chemical nature of the material, different microbial metabolisms may be developed, thus giving rise to both aesthetic and structural damage of the artworks. Aesthetic changes are manifested as pigment discoloration and stains on the surfaces, whereas structural damages are cracking, disintegration, exfoliations and paint blisters (Rajendran and Prasat, 2012). In fact, fungi are able to digest organic matter, altering and weakening those materials, by enzymatic activities, because they excrete a battery of extracellular hydrolytic enzymes, such as cellulases, pectinases, pectolytic enzymes, chitinases, glycosyl hydrolases and proteases (Naji et al., 2014; Osman et al., 2014). In addition, molds produce colored substances that can cause stains and spots on textiles (Abdel-Kareem, 2010).

Most of the reported studies on the characterization of microbial communities associated with biodeterioration phenomena have been focused on damaged stone-monuments, mural paintings, and frescoes (Gorbushina et al., 2004; Scheerer et al., 2009; Capodicasa et al., 2010; Fazio et al., 2015), but investigations on removable paintings on canvas or wooden artworks may also provide a wide range of information on biodeterioration phenomena and microbial diversity, providing useful indications and suggestions on possible restorative and preserving strategies. Moreover, many fungi can be dangerous to people and this has implications for the occupational safety and health of restorers and other museum personnel (Nevalainen and Morawska, 2009; WHO, 2009). For these reasons, the monitoring of microbial contamination on the surface of heritage objects from both a quantitative and a

* Corresponding author at: Department of Biomolecular Sciences, Division of Toxicological, Hygiene and Environmental Sciences, University of Urbino Carlo Bo, Via S. Chiara 27, 61029 Urbino, Italy.
E-mail address: luigia.sabatini@uniurb.it (L. Sabatini).

https://doi.org/10.1016/j.micres.2017.12.003
Received 6 September 2017; Received in revised form 27 November 2017; Accepted 2 December 2017
Available online 08 December 2017
0944-5013/

qualitative point of view is essential to draw a map of microbial contamination and evaluate possible risks. A correct monitoring, with standardized methods and non-destructive techniques, represents the basis for any further prevention strategy.

Montefeltro is an important historical-geographic area in the Marche region, which gave its name to the Montefeltro family, which ruled in the area during the Middle Ages and the Renaissance. The Montefeltro area is characterized by an incredibly landscape but, most importantly, by an amazing cultural heritage and many artists, such as Piero della Francesca, Raffaello Sanzio, blessed this land with wonderful heritage. In the present study, included in a wide project for the restoration and conservation of artworks, the fungal contamination of 64 cultural heritages (wooden, canvases and other typology of artworks), belonging to different collections of Montefeltro area and neighboring areas, was screened in order to obtain more information on the microbial diversity involved in the biodeterioration of cultural heritages. The experimental approach included standard cultivation method in different growth media coupled with direct microscopic observation, when possible, to verify the presence of peculiar fungal structures.

2. Materials and methods

2.1. Artwork description

This study included 64 different artworks (37 wooden artworks, 18 canvases and 9 artworks of different typologies) (Table 1a) of Montefeltro area and neighbouring areas (Fig. 1**)**. The artworks were stored or exposed in different environments (warehouses, museums, private collections, public buildings or outdoor areas) and had ages ranging from XV to XX century (Table 1b). A microbial attack was suspected as the artworks presented visible alterations, colored spots, discolored areas, deposits or patina on the surfaces; for this reason, several samples were taken from areas of about 10 cm^2 in relation to the type and extension of the observed alteration in each artwork (Fig. 2).

2.2. Procedure of sampling

On the basis of the observed changes, sampling was carried out using sterile cotton swabs (VWR, Milan, Italy), wiped on the surface of the sampling areas and transferred in sterile tubes to the laboratory for fungal culturing and identification. In the case of fiber and particulate fragments, samples were directly placed on petri dishes. Areas without visible changes were considered as negative controls.

To verify whether the observed alterations were due to a pre-existing microbial attack or was in place at the time of sampling, a direct microscopic examination was carried out. For this, an adhesive tape was lightly pressed on the sampling area and placed on a slide with a drop of blue lactophenol dye (Sigma-Aldrich, Milan, Italy). Each sample was transferred in laboratory for the microscopic observation with Olympus CX41 (Olympus, Milan, Italy) and stereomicroscope Zeiss STEMI DV4 (Zeiss, Milan, Italy) for the evaluation of typical morphological fungal structures.

2.3. Culturing media and growth conditions

Each collected swab was immersed in sterile tube containing 5 ml of sterile saline solution and vortexed for 1 min at maximum speed; then 0.1 ml was streaked on the surface of different selective media: Sabouraud Dextrose Agar (SDA) (Liofilchem, Roseto degli Abruzzi, Italy), Malt Extract Agar (MEA) (Liofilchem), Czapek Dox Agar (CDA) (Liofilchem). Potato Dextrose Agar (PDA) (Liofilchem) was used in all the subcultures, since it stimulates sporulation and pigmentation (Griffith et al., 2007).

Plates were incubated at 25 °C for 5–7 days and regularly observed. Fungal isolates were identified to genus or species level using biometric parameter (colony diameter) and microscopic features, according to Larone (2002), Samson et al. (2004) and Watanabe (2010).

3. Results

3.1. Occurrence of fungi isolated from different artworks

The data relative to the isolates from each type of artworks (n = 64) are presented in Table 2. A total of 293 swabs were collected and, among these, 2.3% resulted negative to cultural method, while 87.7% were positive for the presence of filamentous fungi, yeasts and Actinomycetes. Totally, 11 genera and 12 different species were confirmed from the cultivated cultures (including 73 strains of *Mycelia sterilia*).

As regards the wooden artworks, a total of 233 strains were isolated from the different cultural media. The most frequent fungi isolated were represented by *Penicillium* spp. (n = 67) with two identified species, *P. chrysogenum* (n = 16) and *P. citrinum* (n = 7), followed by *Aspergillus* genus (n = 59) with six specific species: *A. candidus* (n = 30), *A. versicolor* (n = 5), *A. niger* (n = 3), *A. glaucus* (n = 2) and *A. penicillioides* (n = 2). The third most numerous microorganisms were *Cladosporium cladosporioides* (n = 22), *Mycelia sterilia* (n = 21), *Actinomyces* (n = 14), and *A. alternata* (n = 12).

A total of 266 strains were isolated from canvas artworks. Also in this case, the most frequent isolated fungi were represented by *Penicillium* spp. (n = 112), including *P. chrysogenum* (n = 36) and *P. citrinum* (n = 10), followed by *Aspergillus* group (n = 35) with five specific species: *A. candidus* (n = 15), *A. versicolor* (n = 9), *A. nidulans* (n = 4), *A. niger* (n = 2) and *A. glaucus* (n = 2). In addition, *C. cladosporioides* (n = 30), *Mycelia sterilia* (n = 34) and *A. alternata* (n = 12) were also identified.

The third group included 9 artworks of different typology in term of used materials; in this case, 82 strains were isolated and among these,

Table 1a
List of the different artworks belonging to several collections of Montefeltro area and neighbouring areas.

	Wooden artworks (n = 37)	Canvases (n = 18)	Other typologies (n = 9)
Typologies:	Sculpture (n = 20) Wooden supports (n = 15) Frame (n = 1) Crucifix (n = 1)	Painting (n = 14) Textile (n = 4)	Concrete artwork (n = 2) Hemp artwork (n = 2) Jute artwork (n = 1) Paper artwork (n = 2) Pressed cardboard artwork (n = 1) Chewing gum artwork (n = 1)
Geographical areas:	Montefeltro area (n = 30) Neighbouring areas (n = 7)	Montefeltro area (n = 14) Neighbouring areas (n = 4)	Montefeltro area (n = 6) Neighbouring areas (n = 3)
Observed alterations:	Green, white, brown or black spots Surface deposits	Lacerations, spots	Chromatic alterations, spots
Total Samplig (mean/sample):	153 (4.13)	115 (6.4)	25 (2.7)