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Evaluating the microbiological risk to a contemporary Nigerian painting: Molecular and biodegradative studies

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ABSTRACT

The microbiological risk to a contemporary oil painting on canvas from Nigeria showing stains putatively caused by microorganisms was assessed with non-invasive (nitrocellulose membrane and fungi tape) and microinvasive (fibres) sampling techniques as well as culture-dependent/-independent methods. Optical microscopy observation of fungi-tape showed that there were more surface-associated microorganisms on the discoloured areas of the canvas painting compared to non-discoloured areas. Epifluorescency data demonstrated that the colonizing microorganisms were in an active metabolic state. A higher microbial count obtained from the discoloured fibres implied that the deterioration may be of microbial origin. Bacterial strains isolated were phylogenetically related to members of the phylum Firmicutes and Proteobacteria while the fungi belonged mainly to the phylum Ascomycota. The dominant genera were *Bacillus* and *Ascotricha*, respectively for bacteria and fungi. Cellulolytic assay performed to ascertain the potential of the isolates to degrade cellulose, the major component of canvas, revealed that all the fungal and seven of the bacterial strains could attack cellulose, thereby making them potential biodeteriogens of the artwork. To the best of our knowledge, this is the first report from Sub-Saharan Africa demonstrating the application of molecular techniques to the study of microbial deterioration of a cultural heritage object.

1. Introduction

Oil paintings on canvas are made from organic and inorganic materials which provide suitable ecological niches for growth of various microbial species. Apart from the constituents of the painting, environmental contaminants such as dust, dirt and soot which settle on painted surfaces also serve as sources of nutrients for microorganisms (Ciferri, 1999). However, successful colonization and proliferation of microorganisms on artworks as well as the microbial diversity depend on the prevailing environmental conditions, in particular, temperature and humidity (Dakal and Cameotra, 2012; Sterflinger and Piñar, 2013; Ma et al., 2015).

Microbial growth on canvas paintings results in deterioration of these artworks and is observed as aesthetic (e.g. pigment production), chemical (e.g. enzymes and organic acids production) and physical damage (e. g. hyphal penetration of the painted surface leading to disintegration of the painted layers and degradation of supports) (Ciferri, 1999; Warscheid and Braams, 2000; Crispim and Gaylarde, 2005).

More often than not, microbial deterioration of paintings on canvas begins from the reverse side (López-Miras et al., 2013b), which may be due to high susceptibility of textile material of plant origin to microbial degradation (Szostak-Kotowa, 2004; Kavkler et al., 2015). According to Gutarowska and Michalski (2012), cellulose, the major component of cotton textile and canvas support, is a homopolymer composed of repeating units of D-glucose joined by β-1,4-glucosidic bonds. Biodegradation of cellulose is mediated by the cellulolytic enzyme pool secreted by microorganisms, especially fungi, giving rise to glucose production as well as other metabolic products (Lynd et al., 2002; Duncan et al., 2008; Zyani et al., 2009; Wilson, 2011). These metabolites can be used by other microorganisms in the same community as carbon sources for growth and energy. Therefore, cellulolytic degradation of canvas support will ultimately lead to its depolymerization and

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subsequent loss of fibre's structure. The obverse side of the canvas is generally less susceptible to biodeterioration due to the presence of heavy metals such as lead, zinc or chromium in the pigments (Tiano, 2002; Santos et al., 2009; Pavić et al., 2015). Despite the role of cellulolytic microorganisms in canvas degradation, studies pertaining to their deteriorative potential on oil painting on canvas are relatively scarce.

The initial step in developing an effective conservation strategy requires a sound knowledge of the microbial flora and the nature and types of active indigenous organisms which mediate the deteriorating processes of painting on canvas and other artworks. This will doubtlessly help determine the preventive measures to be taken for appropriate conservation. Although the current application of molecular techniques employed in the identification of microorganisms inhabiting artworks has advanced the knowledge of the surface-associated microbial communities adhering to art objects, classical cultivation method is still significant in the study of biodeterioration of art objects owing to its usefulness in the metabolic study and destructive potential of colonizing microbiota (Schabereiter-Gurtner et al., 2001; González and Saiz-Jiménez, 2005). Therefore, adoption of both classical and molecular techniques is a necessity in biodeterioration studies due to their complementary advantages (Laiz et al., 2003; Palla, 2011; Palla et al., 2013).

Literature is replete with studies pertaining to biodeterioration of frescoes and mural paintings (Rebricova, 1991; Piñar et al., 2001; Gorbushina et al., 2004; Milanesi et al., 2006; Ripka et al., 2006; Cappitelli et al., 2007; Rosado et al., 2014) while very few reports have emerged on canvas paintings (Capodicasa et al., 2010; López-Miras et al., 2013a; Pavić et al., 2015) with none on artworks emanating from Sub-Saharan Africa, particularly, Nigeria. Due to tropical climatic conditions, artworks in Nigeria are subject to various physico-chemical stresses that appear to hasten biotic and abiotic deterioration. Most artworks especially paintings, are prone to damages and hence, rarely last longer than those in developed countries. Museums where some of these materials are kept are in suboptimal conditions and are faced with plethora of problems chief among which is under funding. The present study therefore set out to evaluate the microbiological risk to a contemporary Nigerian painting with the intent of determining microbial abundance and viability on a canvas painting as well as assessment of its microbial community structure with both cultural and genetic fingerprinting approaches. The results show substantial evidence for implication of metabolic activities of microorganisms especially cellulolytic strains as deteriorative agents of artworks.

2. Materials and methods

2.1. Description of artwork and sampling techniques

The artwork used for this study, called 'The Atiliogwu Dancers', was painted in 2000 and kept in the studio of Messrs Niyi Fakeye and Sola Fakeye in Lagos, Nigeria. The painting depicts dancers of Atiliogwu, a traditionally, vigorous dance which is very popular in the south eastern part of Nigeria. Atiliogwu which literally means "is this magic?" combines elements of acrobatics with foot-stomping rhythms. The name came from the belief that Atiliogwu Dancers could dance energetically and exuberantly by the power of magic. The oil painting on canvas (120 cm × 80 cm) depicts two dancers dressed in Nigerian native costume. It showed discolouration putatively caused by microorganisms; the external edges appeared physically damaged and fragile with a felted consistency. Four samples from stained (B4 and B5) and apparently unstained areas (B3 and B6) were collected using three methods: (1) non-destructive adhesive tape strips for microscopic analyses, (2) sterile scalpel for cultural analyses and (3) nitrocellulose membrane for molecular assessment of the community structure. These sampling locations and types of discolouration are shown in Fig. 1. Samples of biological structures from both stained and unstained areas of the artwork were collected with adhesive tape strip (Fungi Tape™, DID Milan, Italy) as described previously (Michaelsen et al., 2009). Microinvasive method was adopted for the collection of samples meant for cultural analyses (Polo et al., 2012). The protocol of Principi et al. (2011) was adapted for the collection of samples for molecular analysis of the microbial community structure. Briefly, nitrocellulose membranes (Sartorius AG, Gottingen, Germany) was pressed gently for 30 s onto the surface of the canvas with the aid of sterile forceps and transferred immediately into tubes with sterile phosphate buffered saline (PBS; Sigma Aldrich, Milan, Italy) for analysis.

2.2. Microscopic analysis

Adhesive tape samples were used for microscopy according to the protocols of Pinzari et al. (2012) with modification. The tape samples were observed for the presence of surface-associated microbial contamination with a phase contrast microscopy after which it was confined with *in situ* frames (1 cm^2; Epperndoff) and stained with 100 μl of 20 μg ml^{-1} fluorescein diacetate (FDA) and incubated for 20 min in the dark. The tape samples were washed with demineralized water three times and observed for the presence of active structures with an epifluorescence microscope (Leica DM 4000 B, Leica Microsystems, Milan, Italy). CoolSNAP CT camera

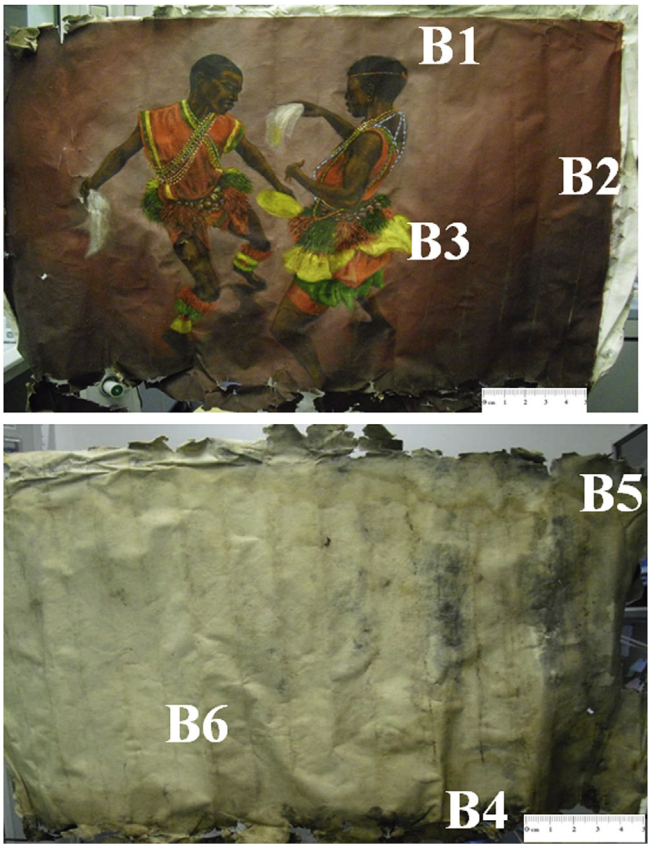

Fig. 1. Location of the sampling areas on the obverse and reverse sides of the canvas painting used for the study. B3, non-discoloured area; B1 and B2, discoloured areas of the observe side; B6, non-discoloured area; B4 and B5, discoloured areas of the reverse side.