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18th Century knowledge on microbial attacks on parchment: Analytical and historical evidence

Cristina Cicero[a], Flavia Pinzari[b,c,*], Fulvio Mercuri[a]

[a] Dipartimento di Ingegneria Industriale, Università degli Studi di Roma Tor Vergata, Via del Politecnico 1, 00133, Rome, Italy
[b] Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Via della Navicella 2-4, 00184, Rome, Italy
[c] Department of Life Sciences, Natural History Museum, Cromwell Road, SW7 5BD, London, UK

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ABSTRACT

A 14th Century illuminated codex underwent an extensive restoration in 1739. The intervention was necessary because the volume was "rotten". Some purple stains are still visible and passed through multiple pages in the thickness of the volume, with a pattern that suggested a resurgence of the infection after the restoration of the manuscript. The unusual appearance of the halos and the light-pink colouring of some of the spots indicated that a topical treatment of some kind could have been carried out. The stains were analysed by non-invasive and micro-invasive methods in order to reveal the chemicals used to disinfect or bleach them and the structural effects of the treatment on the collagen fibres. The chemical compound used to treat the stains of the codex contained sulphur and potassium, it was strongly acidic and in a liquid form. Based on both the results and the knowledge of that time, we hypothesised that in 1739 the restorer was aware of "infectious" nature of the stains and tried to stop the action of microorganisms. The attempt to stop the process would be the testimony of a diffuse knowledge on biodeterioration phenomena, not yet consolidated, but already present.

1. Introduction

Parchment biodeterioration is due to the activity of microorganisms equipped with extracellular enzymes that give them the power to metabolise proteins (Florian, 2007). The species with these capacities are bacteria and fungi that have collagenases capable of cleaving the main proteins of parchment by hydrolysis. Both fungi and bacteria, however, can use as carbon sources also oils and waxes that can be present in skins or be added during manufacturing processes, causing different kinds of damage, with different appearance and degrees of severity (Zyska, 2004; Kraková et al., 2012; Paiva de Carvalho et al., 2016).

The *Liber Regulae S. Spiritus de Saxia* (hereon referred to as "*Liber*") is a 14th Century illuminated codex about the charitable activities of the "Ospedale romano di Santo Spirito in Sassia". The manuscript is preserved at the State Archive in Rome (Italy) and constitutes a testimony of the main acts of charity carried out at the religious hospital such as the relief to sick people, reception of pilgrims and care of orphans and pregnant women (Drossbach and Wolf, 2015; Helas, 2015). The *Liber* is famous for containing the "Rule" of the religious order of the Holy Spirit but also for its unique miniatures; it has been extensively studied and it was recently analysed to evaluate its manufacture and reconstruct the history of its miniatures (Salmi, 1956; Tomei, 2002; Manzari, 2006; Hoffmann, 2008; Mercuri et al., 2018).

An extensive restoration of the volume took place in 1739 by the order of the hospital's preceptor, Antonio Maria Pallavicini (La Cava, 1947; Drossbach and Wolf, 2015). The intervention, as it is reported in an initial page, inserted in the volume during the restoration and titled "*Ad Lectorem*", was necessary because "*characteribus alicubi corrosis, membranis dilaceratis, a temporis iniuria tabefactis coperta fuit*" that is to say that the volume was "putrescent" (Latin word "*tabefactum*"). The biological damage on the *Liber* in the 18th Century must have appeared really serious and extensive since the restoration work requested massive interventions and comprised the reconstruction of page margins. During this restoration the leaf edges were trimmed and mounted on new parchment frames with a few millimetres over-lapping region. The stains affecting the manuscript generated specular patterns on some neighbouring leaves. The trimming and rebinding of the book occurred in 1739 caused a change in the overlap of the stacked leaves, with a consequent loss of matching between the above-mentioned patterns formed before that year. This provided evidence that the main microbial attack was previous to 1739 and that in some leaves the microbial activity continued after the treatment. Since none of the treated stains is

* Corresponding author. Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Via della Navicella 2-4, 00184, Rome, Italy.
E-mail addresses: cristina.cicero@uniroma2.it (C. Cicero), f.pinzari@nhm.ac.uk, flavia.pinzari@crea.gov (F. Pinzari), fulvio.mercuri@uniroma2.it (F. Mercuri).

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present on the outer newer parchment, the treatment must have been performed before or during the restoration (Mercuri et al., 2018). Actually, the margins of some original pages in the manuscript are still showing red or purple spots, nucleated and with peripheral halos, isolated or coalescing. The origin of these stains could be attributed to an attack by proteolytic bacteria that typically produce the metabolites that caused the purple discolouration, particularly disfiguring (Piñar et al., 2014; Migliore et al., 2017).

Piñar et al. (2014) suggested that the species of micro-organisms responsible for this defacing phenomenon could be actinomycete bacteria within the genus *Saccharopolyspora* (Pseudonocardiaceae Family) and hypothesised a connection between the purple spots appearing on ancient parchments and the so-called "red heat", a damage that occurs in protein-based products (i.e. leather) manufactured using marine salt. Some authors recently confirmed the presence of *Saccharopolyspora* spp. on as many documents by means of DNA massive sequencing techniques (Teasdale et al., 2017; Migliore et al., 2017). Migliore et al. (2017), in a study of a defaced parchment roll dated to 1244 A.D. hypothesised a two-phase model mechanism of deterioration, where halophilic Archaea producing purple pigments, colonised parchment and are then succeeded by Gamma-Proteobacteria with fungi as later colonisers. It is known that the presence of chlorides and sulphates can make the parchment microenvironment selective towards halophilic and osmophilic microbial species. Since parchment pH can be strongly alkaline following the liming procedures during manufacturing, its microenvironment can be selective also for haloalkalophiles which are extremophilic microorganisms adapted to both saline and alkaline conditions. In the areas most affected by microorganisms, parchment usually becomes rough, assumes a diffuse staining and a porous appearance, and sometimes becomes perforated like the stains present at the page margins of the *Liber* (Fig. 1a and b). Gallo and Strzelczyk (1971) observed that this kind of microbial growth is generally more significant on the margins and on the first and last pages of the codes, like what happened to the Liber and described in the page added by the restorer in 1739.

The interesting aspect was not so much in the purple spots and the microorganisms that caused them, but the singular aspect of some of the stains. In fact, the unusual appearance of the halos and the light-pink appearance of some of the spots (Fig. 1a), suggested that a topical treatment of some kind could have been carried out during the restoration dating back to the 18th Century. The aims in this study were therefore to prove the treatments occurred in the past and to confirm their nature with scientific methods and the available historical documentation. The stains were analysed by non-invasive and micro-invasive methods in order to reveal the possible use of chemicals to disinfect or bleach them and the structural effects of this hypothesised treatment on the collagen fibres.

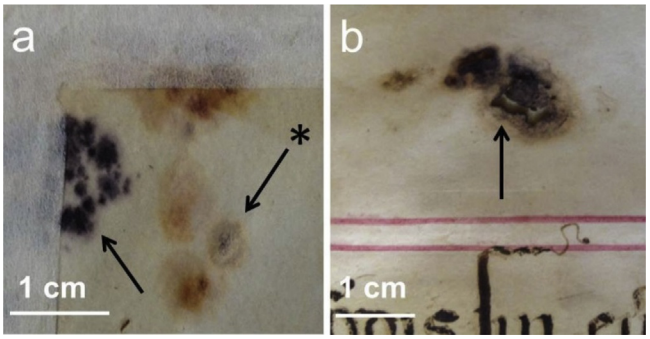

Fig. 1. *Liber Regulae*; a) treated stains (arrow with asterisk) and un-treated stains; b) un-treated stain with a perforated core, due to collagenolytic activity of bacteria.

2. Materials and methods

2.1. Sampling

Fragments of parchment of ~0.5–2 mm in diameter (Supplementary Fig. 1) were sampled according to Puchinger and Stachelberger (2002). The micro-samples so obtained from the *Liber* and analysed with SEM-EDS were 8 (Supplementary Fig. 1). Three from un-treated spots (from folia 100 and 245) and five from treated spots (from folia 25, 28, 222). The EDS data obtained from these samples were grouped in 2 categories: un-treated and treated. These were further grouped in 2 sub-categories (surface and fibre), according to where the EDS probe was directed during the analysis (respectively on the surface of parchment, where sizing was still visible with BSD-SEM imaging, and on bare collagen fibres exposed as a consequence of a partial split or etching of the sample). The captions of these four groups of data were as follows: un-treated fibre (ut_f), treated fibre (t_f), un-treated surface (ut_s), treated surface (t_s).

Photomicrographs were taken of all locations before and after sampling. The core samples were used for low vacuum observations with a variable pressure SEM coupled with backscattered electron diffraction (VP-BSD SEM), and in high-vacuum SEM observations (HV-SEM), after metallization, to examine the fibres morphology and the remnants of microbial attack at high magnification.

2.2. Stereomicroscopy

A Leica MZ16 stereoscopic microscope fitted with low temperature fibre optic lighting was used to examine the samples and mount them on stubs before SEM-EDS analysis. The system was equipped with a digital camera connected to a computer with software that allowed composition of multifocal images (Leica Application Suite, LAS, Leica Microsystems GmbH Wetzlar, Germany).

2.3. Scanning electron microscopy (SEM)-energy dispersive X-ray spectroscopy (EDS)

Parchment samples were examined uncoated using a VP-SEM (EVO50, Carl-Zeiss Electron Microscopy, Welwyn Garden City, Hertfordshire, UK) equipped with detectors for backscattered electrons (BSE) and secondary electrons (SE). Chemical analysis was performed by means of EDS (INCA 250, Oxford Instruments, Abingdon-on-Thames, UK). The SEM was fitted with a tungsten filament and operated at 20 keV, with an average working distance of 12.5 mm, and with a chamber pressure between 30 and 150 Pa, chosen according to the need for maintenance of parchment fibres original structure. The EDS analyses were calibrated using standards ($CaCO_3$, SiO_2, Albite, MgO, Al_2O_3, GaP, FeS_2, Wollastonite, MAD-10 Feldspar, Ti and Fe) and the conventional ZAF correction (for atomic number Z, absorption and fluorescence) was applied, integrated into the Oxford INCA 250 microanalysis package used. Nitrogen was calculated in EDS spectra according to Gazulla et al. (2013). The samples, after being observed with SEM in Variable Pressure mode, were metallised with gold in a Baltec Sputter Coater for further analysis in High Vacuum mode. The sputtering was performed under an Argon gas flow, at 50 mm working distance with 0.05 mbar of pressure and a current of 40 mA, for 60 s to obtain a film of gold of about 15 nm.

2.4. Statistical analysis

The EDS measurements were analysed using statistical tests to evaluate the relationships between the measured element, and the significance of the differences between samples and specific areas within each sample. One-way analysis of the variance (ANOVA) was applied, and the significance of the differences was tested at 95% confidence. ANOVA was followed by a post-hoc analysis. Fisher's LSD