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Bio-deterioration and Bio-degradation of Collagen-based Culture Heritage: Parchment and Archeological Leather

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Abstract

As natural collagen-based cultural relics, parchment and leather are susceptible to the storage environment, such as temperature, relative humidity, pollutants and microorganisms, resulting in the deterioration of the main components in them including collagen, keratin, tannins, lipids, oils and tanning regents. Obvious changes might take place in the appearance, composition and internal structure, accompanying with the decrease in physical properties such as thermal stability, flexibility and tensile strength. Biodeterioration is one of the main factors affecting the long-term storage of parchment and leather artifacts, particularly taking place at high relative humidity environment because of the proliferation and growth of bacterial or fungi. This present review is focused on the common microbial communities on the parchment and leather artifacts. The biodeterioration mechanism is discussed, which is expected to provide guidance for the future research of collagen-based cultural relics.

Introduction

As an abundantly available natural resource, animal skins/hides have been being used as the raw materials of parchment and leather products to meet the needs for survival or daily life, even at the beginning of the human civilization. There are several stages in the conversion from skin/hides into leathers including beamhouse, tanning and finishing, while the beamhouse is similar to the parchment preparation.[1] The epidermis, the keratin-rich outer layer of the skin, the hypodermis and the subcutaneous fatty tissue are generally removed in the beamhouse.[2] By tanning, the skins/hides are transformed into leathers with excellent thermal stability, putrefactive resistance, as well as good mechanical properties. Parchments and leathers have been with us for a long time in the human civilization, and many culture relics are being unearthed and handed down, which are the precious historical heritages for us to study the civilization of ancient societies. So how to preserve them properly to ensure their longevity is therefore of great significance.

The damages of parchment and leather artifacts caused by the environment factors can be summarized as the physical damages, chemical damages, biological damages, and others. For biological damage, a variety of microorganisms might pose a huge threat to parchment and leather artifacts because the collagen, keratin, tannin, lipids, oils and other substances in them could be ideal nutrient sources to well support the growth and proliferation of fungal and bacterial in the environment with high relative humidity. Hides or leathers might be damaged by bacteria, which are mainly responsible for the decomposition of the untanned collagen in raw hides and during soaking. The fungi might thrive on tanned leathers containing carbohydrates, fats and proteins. Consequently, aesthetical changes occurred on the surfaces of parchment and leather objects, such as discoloration or biofilms. The structure of the leathers might be weakened and complete destruction might even take place.[3] One of the most common aesthetical damages caused by microorganism is the formation of purple stain or pot due to the fungal pigments.[4] Besides, the metabolization of microorganisms generates extracellular enzymes to hydrolyze the collagen.[5] In Fig. 1, microorganisms are able to inhabit in and even decay the historical art objects such as books, leather artifacts, and parchment. Particular attention was paid to the parchment document of 505 mm in height and 470 mm in width, which was tanned on both sides and partially glued with patches of parchment and paper during the conservation with certain microbial defects on the upper half and left side (Fig. 1E).

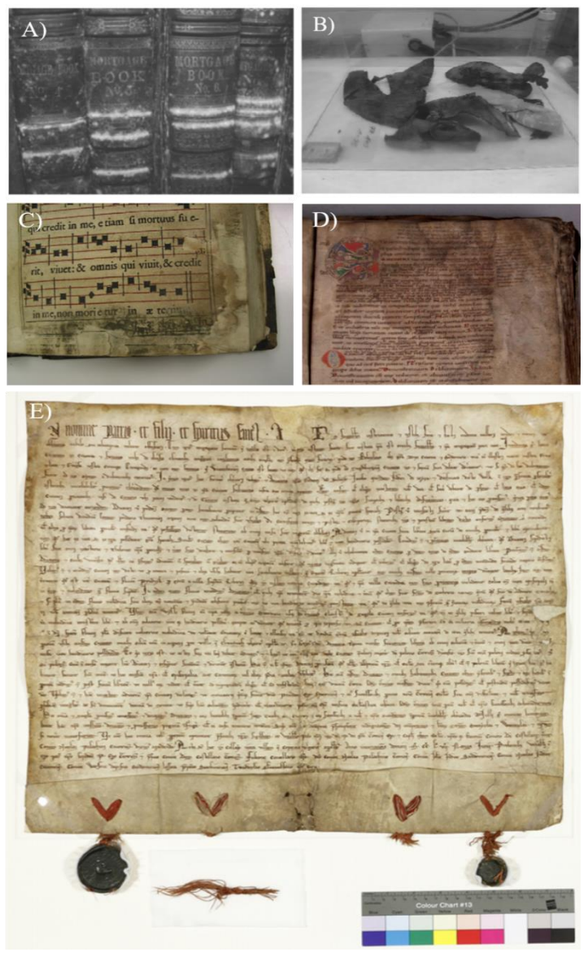

Figure 1 The microbial attack on A) book blinding leathers preserved in European museum, B) shoe fragments unearthed in House Page, Germany, C) historical book, D) text in National Library of the Czech Republic and E) A parchment document from the 13th century—the incorporation charter for the city of Krakow.

Identification of Microbial Communities

Identification of microbial communities is crucial for the conservation of valuable organic objects including the collagen-based cultural relics such as parchments and leathers. Totally, the microbial that is undesirable found on parchment and leather artifacts are divided into bacteria and fungi. 35 bacterial strains belonging to various genera (*Streptomyces, Bacillus, Pseudomonas, Serratia* etc.) were screened for their ability of growth in minimal medium containing bovine leather, ovine leather or wool as unique carbon or nitrogen sources. Fungi is classified into yeasts, moulds, mushrooms and toadstools. Even in 1839, it was known that there existed some fungi capable of attacking keratinized tissues, although, at that time, only dermatophytes were recognized. As in 2009, 299 fungi belonging to 100 genera were reported about the microbiological deterioration of keratinous substrates. The most common genera *are Aspergillus, Penicillium, Chrysosporium, Fusarium, Microsporum, Trichophyton,* and *Acremonium*.

In order to know the physiological characteristics of pure isolated strains and to develop the metabolic studies, two traditional methodologies of cultivation and microscopy were usually applied. However, these classical methods lead to an underestimation of the composition of the colonizing microflora, one of which is that only a small proportion of microorganism could be isolated. In the last few decades, culture-independent methods such as denaturing gradient gel electrophoresis (DGGE) and clone library have been developed and widely applied in the study of microbial communities on biodeteriorated cultural materials.

Biodeterioration of Leather

(1) Biodeterioration of Vegetable Tannins

Tannins are water-soluble phenolic compounds usually extracted from plant by hot water. Generally, tannins could retard the decomposition rate of vegetable matter by preventing the biodegradation enzymes from attacking organism. For this reason, tannins have long been considered as inhibitors of most microorganisms. For example, they could act as strong inhibitors of Azotobacter and nitrogen-fixing, nitrifying and sulfate-reducing bacteria. Soil bacteria such as Rhizobium are also often sensitive to tannins. Fungi, such as Fusariumv, Verticillium, Aspergillus, AUernari, as well as yeasts can be inhibited by tannins too. Despite the antimicrobial properties of tannins, many fungi, bacteria and yeast are quite resistant to tannins and tannins might even serve as their carbon and energy sources. In particular, Aspergillus spp. and Penicillium spp. have found application in the biotransformation of tannins for leather tanning. The biodeterioration of natural tannins in the environment is mainly associated with fungi rather than bacteria. The condensed tannins are more recalcitrant to biodeterioration than the hydrolyzed ones.

(2) Biodeterioration of Collagen in Leather

Collagen is predominantly stabilized by non-covalent interactions and conjugated with polysaccharide. Covington et al. found that the binding force between tanning agent and collagen had strong anti-microbial decomposition property, which could be completely biodegraded after thermal deformation. However, Israelroming et al demonstrated that microorganisms were able to biosynthesize collagenolytic enzymes as well as keratinolytic enzymes to take part in leather biodererioration.

(3) Biodeterioration of Non-Collagen Component in Leather

The invasion of non-collagenous components by microorganisms to collagen-based cultural relics, especially for ancient leather artifacts is critical should not be ignored. The non-collagen components in leather include keratin, elastic fiber, proteoglycan, fat, muscle fiber and so on, among which, keratins form a group of highly specialized proteins produced in epithelial cells of vertebrates.

Biodeterioration of Parchment

Similar to other materials like paper and leather being able to carry writing, parchment is subject to biodeterioration. Parchment exists as a post-mortem tissue. The in vivo mechanisms of collagen turnover and repair that existed in living skin are no longer present. With no repair mechanism in place, the collagen present in skin is subjected to irreversible degradation. Factors that can bring about collagen degradation in parchment include biological sources of degradation such as bacteria, fungi and rodents.Parchment has a pH of between six and eight, and is a source of nutrition for many microorganisms. Microbial enzymatic degradation of the organic components takes place in the process. The main organic component, for paper is cellulose, while collagen is the main organic component for parchment and leather.

Parchment biodeterioration is due to the activity of microorganisms with extracellular enzymes to endow them with the ability to metabolize proteins. The species with these capacities are bacteria and fungi, which are capable of cleaving the main proteins of parchment by hydrolysis by the collagenases. Both fungi and bacteria might use the carbon sources of oils and waxes in parchments, which are originated in the skins/hides or introduced during manufacturing processes, causing different kinds of damage, with different appearance and degrees of severity.

The behavior of microbials attacking to collagen in parchment could be summarized in three paths: molecular denaturation, hydrolysis and oxidation. The level of oxidation of collagen in parchment can be assessed by measuring the ratio of basic to acidic amino acids (B/A ratio). In new collagen this ratio is 0.69, but as the collagen undergoes oxidative change, this ratio will decrease. Historic parchments have shown B/A ratios as low as 0.5.

Biodeterioration of Parchment

The reported works on the biological damage of collagen-based cultural relics of parchment and leather were reviewed, with the microbial species and the bioterioration mechanism specially focused. Considering the environment that collagen-based cultural relics including parchment and ancient leather are usually kept in, either in the burial conditions or in the museums, seriously threatening from various microorganism contamination have to be faced for centuries. So the studies on the microbial deterioration and the development of appropriate restoration of collagen-based cultural are of great importance. However, no systematic studies and few applications could be found at all.

The main research in the future should be specially focused on the biodeterioration mechanism of collagen-based cultural relics from viewpoints of chemical reaction, structure formation, in order to scientifically design the identification, restoration and preservation of parchment and ancient leather. The detection and analysis techniques that are capable of disclosing valuable information should be further developed, such as both recent and past microbial contamination, the change of environmental conditions, and particular events happened to the ancient objects. A long-term and effective protection and preservation for collagen-based cultural relics is needed and the storage steps should also be simplified to avoid secondary damage during processing.

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