



# PhD in Evolutionary Biology and Ecology



## CUM GRANO SALIS: ASSESSING THE CAUSES OF THE PURPLE SPOTS ON HISTORICAL PARCHMENTS

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Historical parchments, used as writing material for many centuries, are causes of great concern for librarians and archivists all over the world because parchments are easily involved in biodeterioration processes. Microbial damage often leads to the loss of parchment's readability, representing a significant issue for the cultural heritage. A frequent parchment's alteration is due to isolated or coalescent purple spots with a nucleated peripheral halo, more crowded on the flesh side. Similar features of damage, known as red heat deterioration, are observed on modern brine cured hides. Very recently, a study was performed on a purple damaged parchment roll dated back 1244 A.D. the A.A. Arm. I-XVIII 3328, belonging to the oldest collection of the Vatican Secret Archive (Fondo "Archivum Arcis"), by comparing uncolored undamaged and purple damaged areas of the same document. As a whole, the study gave interesting results to hypothesize a model of biodeterioration, consisting of a microbial succession acting in two main phases: the first one, common to all the damaged parchments, is characterized by halophilic and halotolerant bacteria fostered by the salty environment within the parchment maybe induced by brining of the hides; the second one, changing with the individual history of each parchment, determines the identity of its colonizers. The design of this model was pivotal to the research work performed during the 1<sup>st</sup> year of this PhD, in collaboration with the Vatican Secret Archive. Three documents, belonging to a collection of dramatically damaged parchments archived as "Faldone Patrizi A 19" (dated XVI-XVII century A.D.), were analyzed through a multidisciplinary approach, including three updated technologies: (i) Next Generation Sequencing (NGS, Illumina) to describe the microbial communities colonizing the damaged and undamaged areas, (ii) RAMAN spectroscopy to analyze the purple pigments, (iii) Light Transmitted Analysis (LTA) to evaluate the kind and entity of the damage to native collagen.

The metagenomic data obtained from NGS revealed DNA sequences belonging to *Halobacterium salinarum* mainly in the undamaged areas. RAMAN spectroscopy detected pigments within the purple spots, mainly bacteriorhodopsine/ rhodopsin-like pigments, a purple transmembrane protein containing retinal and present in Halobacteria. The LTA technique revealed extremely damaged collagen structures in both damaged and undamaged areas of the parchments. In the light of these data, the microbial succession model described before was validated.

During the 2<sup>nd</sup> year of this PhD, purple damaged from modern brine-cured hides will be analyzed with the same multidisciplinary approach described before, in order to assess the responsible agent of red heat deterioration. Moreover, to demonstrate the colonization model that has inspired this project, a home-made parchment will be produced through a traditional procedure trying, in order to reproduce the salinity conditions within the parchment, to allow the growth of rhodopsine-producing microorganisms. *Cum grano salis* the project aims to detect and to prove the causes of the purple damage, in order to start any possible new restoration strategy to bring back historical parchments to their original beauty.