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Integrative Applications of Microbial Biotechnology to Cultural Heritages Conservation and Promotion

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Abstract. In the last decades a number of microbial biotechnology applications have been put into practice for the conservation of cultural heritages. Soft approaches to the bio-cleaning and bio-restoration of cultural heritage are in constant demand in addition to deep understanding of microbial bio-deterioration. Recent research has addressed this issue by different perspectives, highlighting how separate disciplines such biology, microbiology, biochemistry are taking the problem from each own view. However, in the last years the integration of these disciplines have increased the range of tools for conservation and promotion. This commentary shows recent examples of concrete exploitation of the integration of these applications in the field of cultural heritage. Current application of omics techniques and their use for restorers and conservators still rest on the assumption that this omics revolution will be – at some point, important. This commentary includes combination of evidences showing we are a step further: having used a combination of key papers in support, this perspective shows how such applications are already bringing important tools for conservators. Taken together, these sources of evidence are provocative and at the same time similarly stimulating and convincing.

Keywords: biodeterioration, cultural heritage, metagenomics, proteomics, metabolomics, conservation

1. Cases studies in Cultural Heritage conservation

Conservation of Cultural Heritage and Microbiology have always been deeply linked each other. The reason is clear: one of the most destructive degradation of artworks is the microbial degradation which affects artworks and ancient objects made by any type of material, such as stone, paper, textile, wood, leader and combination of them [13, 17]. In addition to the studies on degradation, biotechnological applications have been developed for a dual objective: to apply new biotechnologies for the study of deterioration and to apply biotechnology for the restoration/conservation of artworks [9]. The application of biotechnology has found important development on a number of case-study, ranging from bio-consolidation of stone to cleaning of surfaces by using bacteria [3, 11, 12, 15].

Recently, the development of new omics techniques has provided deeper understanding of the meta-tassonomic and meta-genomic pools of degrading microbial communities [7, 8, 10]. The suffix omics indicates recent high-throughput techniques aimed at the detection of biomolecules of a specific biological system [5]. Such technologies are mainly used to analyze genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). In particular, metagenomics and next-generation sequencing (NGS) techniques have had a huge impact on any aspect of modern microbiology.

In the last decade a continue increase of papers published in this field reflects the continuous interest in understanding the reason of degradation and to apply biotechnological solution to conserve, and in some cases to restore artworks [10] (Figure 1).

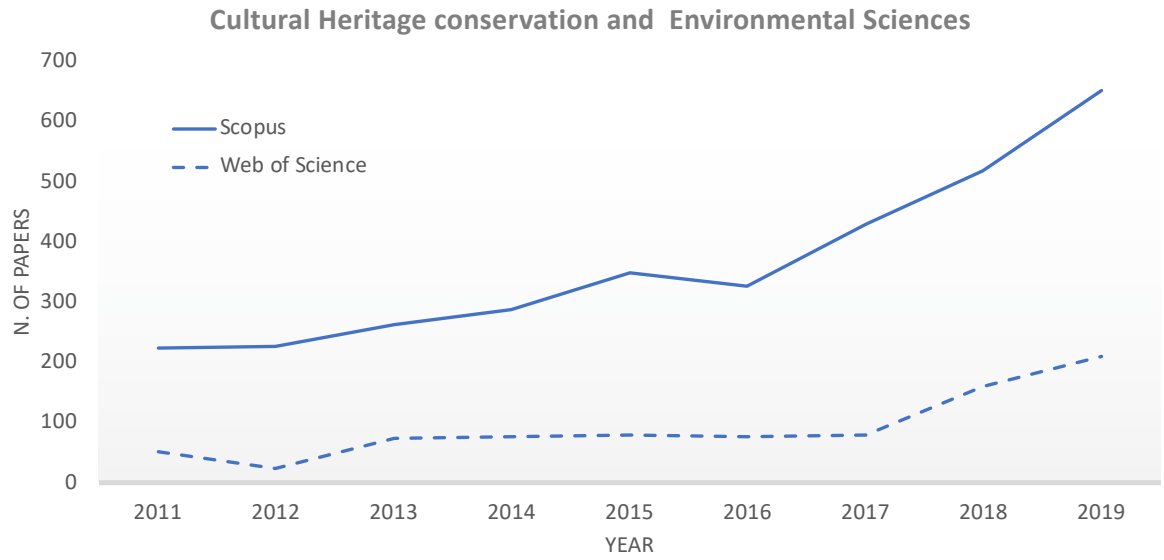

Figure 1. Number of papers published in the field of Cultural Heritage Conservation and Life Sciences in peer-reviewed journals tier 1 or 2. Data obtained from Scopus and Web of Sciences.

Recently cultivable and not cultivable approaches have led to the understanding of deep interaction of the microbial community and the artwork. The study of the ecology of such system has provided important information, not only about degradation, but also as bio-restoration applications. In the last years a few research groups have started to approach the problem in a different manner, by including a comprehensive vision of the omics techniques for a more exhaustive identification of the biodegradation. The application of genomics, metagenomics (non-cultivable methods) to cultivable methods has been expanded through a number of new technologies such as metabolomics and proteomics techniques [6, 8, 13]. In this manuscript are reported concrete exploitation of the integration of these applications in the field of cultural heritage.

2. Revealing biodeterioration

A first paper using this integrative approach has been published by Gutarowska and collaborators in 2015 [4]. The complete study provides a modern approach to the degradation problem, studying the biodegradation of bricks and wood of the Auschwitz II-Birkenau concentration camp (1940-1945) in Oświecim, Poland. The site has an exceptional memorial value and it is particularly vulnerable to microbial degradation since it expands on 171 ha, and includes 155 barracks, 300 relics, 35 watchtowers. All the buildings are made by a combination of wood and bricks and are exposed outdoor, requiring severe monitoring to reduce and prevent onset of bio-deterioration. Products of degradation were studied by using ultra-high performance liquid chromatography (UPLC) coupled to high-resolution mass spectrometry (HRMS) and quadrupole time-of-flight mass spectrometry (QToF-MS). This allowed the detection of active metabolic pathways and degrading chemicals released by the microbiome resilient on bricks and wood. The metabolome was characterized via mass spectrometry and revealed about 1800 metabolites that were released on the artwork by the microbiome [4]. Among them a number of organic acids contributing to the degradation were detected: acetic acid, octanoic acids, decanoic acids, dodecanoic acids, traumatic acid, 2,4-pentadienoic acid, octene-2,4-diynoic acid, 2-ketoglucuronic [to cite some of them and about 120 different organic acids were identified] [4]. Acetic acid and 2-ketoglucuronic acid have been previously identified as corroding chemicals of stone material and they were distributed in both wood and brick samples [14]. When metabolites were associated to pathways and clustered by sample site, CO_2 assimilation and degradation of chlorophyll were identified in the brick specimens. In addition to

acids, the production of antibiotics is also interesting: mycinamicin VII (both bricks and wood), megalomicin C1 (only on wood) were detected, providing information about the ecology on such secondary metabolites released by the microbial community [4]. We can speculate that the identification of such antibiotics' producers could help in controlling cellulolytic organisms, arguing that their removal could even speed cellulose degradation. In addition, Illumina sequencing (MiSeq) was used to unravel the DNA profiling of microbial communities demonstrating high biodiversity [4]. Total DNA was extracted and sequenced by fragments of wood and bricks. Authors showed that microbial diversity of brick specimens was dominated by algae and cyanobacteria, while wood was mainly colonized by fungi [4]. It has to be noted that the study also explores how environmental fluctuations affect this complex network [4]. However, these studies were performed in an environmental chamber and results would possibly deviate from real environmental conditions. Finally, field emission scan electron microscopy (FESEM) imaging was used to reveal the deterioration on material, confirming the microbial leaching problem by visualization of *Gloeocapsa* cyanobacterial clusters of μm-sized pits in the supporting structure. It is clear that all these data are important to understand the ecology of the system before to implement an intervention of cleaning.
A similar modern approach was proposed by Adamik and collaborators in 2017 and 2018 [1, 2]. The studies explored salt efflorescence from a 19th century building and on an *ad hoc* built ceramic brick with known physical and chemical properties. Salt efflorescence is a common problem to any stone material, in particular ancient walls and frescos. Halophilic microorganisms, such as the genus *Rubrobacter* are known to be responsible for discoloration on efflorescence. The use of the next generation sequencing approach led to a remarkable expansion of the known genera identified from this damage [2]. In conjunction with this, an untargeted metabolomics approach of the halophilic community on building materials has, for the first time, contributed to revealing patters of metabolic responses of these microorganisms with the substrate [2]. This strategy could be used to study how osmolytes can prevent the release of pigments, including β-carotene, particularly present in KCl efflorescence and contributing to the aesthetical damage. In addition, corrosion was also detected because of the release of acetic acid (in part to counter the alkaline environment) [2].

3. Blast from the past

Another very interesting example of promotion of antique manuscripts by using a combination of omics techniques and biochemistry has been recently reviewed in a paper with an evocative title "What Sherlock sorely missed: the EVA technology for cultural heritage exploration" [16]. The methods exploited clinic-chemical investigations on documents related to our cultural heritage and detecting potential pathologies left on the pages written by famous authors [16]. A few examples: cause of death by Bulgakov the original manuscript of the novel Master y Margarita; presence of the bacteria responsible for plague on the death registries of the Lazaretto in the 1630 Milano plague; couses of microbial infection on the shirt worn by A. Chekhov in his death bed; traces of infectious diseases on the memoirs of G. Casanova. The ethylene vinyl acetate (EVA) method consists of a plastic polymer in which are applied strong cation and anion resins, through that it is possible to capture proteins and metabolites for mass spectrometry identification. The new application is a revealing new secret from antique manuscript, habits of the previous famous owner, diseases, even type of foods eaten. In the past, sample recollection has been done via surface scraping and erasing, which is a descriptive method. Today, the application of this resin could identify microbial signatures also for studies on restoration and microbial degradation, without any damage to the precious artefacts.

From these pioneering papers, it is clear that the integration of different techniques is pivotal for the understanding of the biodeterioration on a higher dimension which includes: i) improved risk assessment through the identification of indicators metabolites/microorganisms (biomarkers) [3]. ii) A better understanding of the biological damage, its biological course and prediction of the extension of aesthetical and mechanical detriment could help conservators for more focused actions, including

programmatic interventions according with seasons, sun irradiation, temperature and humidity, supporting appropriate choice of coating chemicals and biocides. iii) Improved cleaning procedures of pigments and consolidating methods to counter leaching or microbial degradation.
These integrated approaches will open a gold era to cope with the microbial biodeterioration and biorestoration. The only missing component is a deeper integration between conservators and scientists, this is already happening in the chemistry and physic disciplines, but it is still in its infancy for the biochemistry and microbiology applications.

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