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Characterization of the main colonizer and biogenic pigments present in the red biofilm from La Galea Fortress sandstone by means of microscopic observations and Raman imaging

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ABSTRACT

This work aims to understand the nature of the main colonizer and the composition of the biogenic compounds present in a reddish biofilm located on the sandstone from the Northside of La Galea Fortress (Getxo, North of Spain). For that purpose, microscopic observations of the main colonizer and the biofilm were conducted using Phase Contrast Microscope and a Scanning Electron microscope. These observations allowed to identify the *Trentepohlia* algae as the main colonizer responsible for the reddish biofilm formation. In order to determine the composition of the organic compounds present in the *Trentepohlia* algae and those excreted by them, point-by-point and Raman imaging using confocal Raman microscope were applied. These analyses allowed to identify not only β-carotene as the main biogenic pigment but also additional carotenoids such as bacterioruberin. Moreover, the identification of scytonemin suggests the presence of cyanobacteria on the biofilm.

1. Introduction

The main objective of the all the researchers and professionals working with items belonging to the Cultural Heritage is to try to preserve the history around monuments and historic buildings with more relevance. For centuries, sandstone has been one of the most used materials to construct historical buildings. This material exposed to the open air (atmosphere) can suffer biological colonization. One of the main consequences of this biological growing is the formation of biofilms [1]. In the literature many authors pointed out that the biodeterioration process can promote physical and mechanical stress, chemical changes in the composition of stone substrates and esthetical changes in the facades of constructions [2]. The micro-organisms that promote these kinds of biodeterioration processes generally are algae, bacteria, cyanobacteria, fungi, mosses, lichens, higher plants, etc. [3–6].

The first responsible for stone deterioration are the phototropic microorganisms are the phototropic microorganisms. In some cases, after the growing of these kinds of microorganism, harmful invasions by mosses and vascular plants can be unchained [7]. The invasion by vascular plants is the most critical step in the destruction of monuments, constructions, buildings, etc. made of sandstone [8]. The pioneering microorganisms such as cyanobacteria and algae are able to produce biofilms that retain water and entrap dust, soil particles, spores and seeds; and in some critical cases, they are also responsible of crack and fissure formation [9].

Another factor to consider is the facade orientation of the building where the colonization is placed. Normally, these microorganisms (autotrophs and phototrophs) colonize the north facades of buildings made of sandstones, in which the orientation is more influenced by the rain and wind [10].

In some particular cases, microorganisms colonizing in stone and other building material surfaces can act as a bioprotection for the material itself. These biological biofilms can act as a protective barrier against environmental stressors [11]. In the literature there are many works dealing with the bioprotection and related applications to extend the life of historical buildings [12,13].

Regarding the colonizers, some microorganisms can grow in hostile environments. For this reason, the probability of biological growing depends on several factors. One of the main factors could be the environmental conditions on which a specific type of microorganism may grow [14]. In this way, the relative humidity percentage (% RH) is one of the main environmental factors considered in the growing of microorganisms on stone surfaces [15].

The physicochemical parameters of the substrate have also high influence on the microorganisms growing and biofilm formation process.

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Depending on the physical parameters of the rock, i.e., permeability, porosity and also on the conservation state of the stone (i.e. small cracks, fissures, too abraded stones) different organisms can grow [16]. The nutrients for the colonizers can come from the stone substrate itself [17] and also from airborne particulate matter deposition coming from the atmosphere (i.e. crustal particulate matter coming from the erosion of surrounding stones, airborne particulate matter carried by marine aerosol).

In the literature there are numerous works about the problem of microorganism colonizations in different types of materials. Sometimes, marble and limestone can be intensely bio-erosioned by different phenomena like epilithic and endolithic forms of sponges. This observation has been ascertained by using X-ray diffraction for matrix characterization and SEM for the identification of sponges [18]. In other cases, hot spring travertine material biocolonizations were characterized using FEG-SEM, SEM-EDS and Raman spectroscopy [19]. In other works, the influence of biocolonizations on ceramic was also studied, observing the growth rate for different colonizers using Phase Contrast Microscopy and monitoring the ceramic bioreceptivity by 2D scanning for the colonizer surface using a Pulse-Amplitude Modulated Fluorometer and quantifying the emitted fluorescence by different colonizers [7].

According to the growing of algae biofilms in sandstones placed on constructions, studies that carried out measures over sandstone colonized sub-surface in order to see the moisture movements and conditions using 2D electrical resistivity tomography (ERT) and observing that some biofilms can act as bioprotector against different stressors [11] can be found in the literature.

The main objective of this work is to characterize the nature of the main colonizer responsible for the reddish biofilm formation in the sandstone from La Galea Fortress (Getxo, North of Spain). For this purpose, microscopic observations using Phase Contrast microscope and SEM were conducted. Moreover, an in-depth characterization of the biogenic pigments present on the biofilm was conducted by means of micro-Raman spectroscopy using point-by-point and imaging analysis strategies. Finally, and in order to observe if the composition of the sandstone from this fortress can be a good substrate for the colonizers responsible for the biofilm formation, an elemental and molecular characterization of the sandstone which supports the biofilm was conducted using SEM-EDS and micro-Raman spectroscopy.

2. Materials and methods

2.1. La Galea Fortress and the surrounding climatology

La Galea Fortress is located in the cliff overlooking the Bay of Agra, in Getxo (north of Spain) and about 80 m above sea level. The Fortress is an 18th century military building and took the place of an old watchtower that already existed in the 16th century (Fig. 1A). It has masonry and ashlar walls, 14 embrasures for cannons (originally they were 18) and it is surrounded by a moat on the land side. The building hosted several storehouses to keep arsenals, as well as installations for the troops. The most important part of the fortress is the principal tower (Fig. 1A, B, C). The tower of the military fortresses was the most important part of this kind of fortifications, where the soldiers observed possible threats. When its military function was discarded at the end of the 19th century, it was used as a lighthouse, until the middle of the 20th century.

The climatic conditions of the location where La Galea fortress is immersed are the following: average temperature of 18.3 °C, average minimum temperature of 7 °C and average maximum temperature of 20.3 °C. Moreover, the annual precipitations are around 1200 mm in which at least, during 128 days rainfall above 1 mm are recorded. The annual average relative humidity is around 72%, but in some periods the humidity reaches 90% or higher values. The annual sunshine hours are 1532 and the average wind speed is 5.6 kts, where the most common directions of wind are to the north-northeast and south-southeast components [20].

2.2. Sampling procedure

The conservation state of the sandstone from the fortress tower is very poor, especially the Southside of the tower (Fig. S1, S2). Alveolization and disintegration phenomena are clearly visible. In some area, certain sandstone blocks are almost disappearing (see details of the disintegration and loosing of the sandstone in Fig. S1, S2). The towerside of the fortress that only shows red biofilms is the north facade. This is the only orientation which shows that sandstone blocks are in a good state of conservation (Fig. 1B, C). In the literature, many authors point out that these kinds of colonizations normally occur facing north and in areas with high moisture content [21]. In this facade, several sandstone fragments with red biofilms were collected. To carry

Fig. 1. (A) General view of La Galea Fortress, (B) red biofilm colonizing the sandstone from the Northside of the Fortress tower and (C) general view of the Northside of the tower showing the red biofilm on the sandstone blocks.