Use of natural products for the microorganisms removal from stone church cultural heritage

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Introduction

In recent decades, scientific research has initiated important studies in the field of restoration and conservation of cultural heritage in which biotechnologies, i.e. the application of technologies that use biological systems, living organisms and their products, are predominant. Being able to apply the principles of "Green Chemistry" to restoration means using biocompatible materials and innovative techniques to protect the health of operators and reduce environmental damage due to the use of polluting products. Biodeterioration of stone assets is a long term process produced by the microbial communities living and developing on the surface which release organic and inorganic acids, being involved in redox processes of the matrices constituents (bio-corrosion). The understanding of the complex interactions between colonizing organisms themselves, environmental pollutants and chemical degradation processes represent the necessary basis for the diagnostics of monuments degradation status and their specific maintenance. In recent years, the study of natural biocides able to selectively remove biological contamination from stone surfaces has intensified [1,2].

Aim of the work

In this work, the biocidal activity of glycoalkaloids (GAs) was investigated. The study site was represented by the stone church of San Pietro Barisano (part of the UNESCO heritage) located in Matera (southern Italy) and specifically by the hypogeum of the structure, where significant biodiversity was found. The first experiments were focused on the solely use of GAS extracts to verify their action in hypogeal heritages. Then GAs were inserted on proper gelling agents as supporters. The comparison of different polyvinyl alcohol (PVA)_based hydrogels is in progress. It can be anticipated that their use is suitable to maintain the glycoalkaloids activity and offer the possibility to be easily applied to the deteriorated stone artifacts and removed afterwards.

Materials and Methods

Glycoalkaloids were extracted from unripe berries of *S. nigrum* by using the method of Cataldi et al. [3]. For our experiment we used extracts from *S. nigrum*, containing two main Gas, solamargine and solasonine, and other less abudant components [4].
The first phase of the work was the investigation of biological communities present on the stone surface favored by both the microclimate inside the church and the important infiltrations of water.
The second phase of the work consisted in the sampling, isolation, identification and characterization of fungal species, present on the deteriorated surface as described by Mang et al. [5]. Another interesting part of the work concerned the chemical characterization of calcarenite samples performed by surface analysis using X-rays photoelectron spectroscopy (XPS), before and after the cleaning treatments with GAs.
Finally, the composition of the biogels to be used as a support for the glycoalkaloids extracted from the *S. nigrum* plant, is under optimization in order to evaluate the efficacy of the biocidal activity against the identified microorganisms and to verify any alteration due to the gel application.

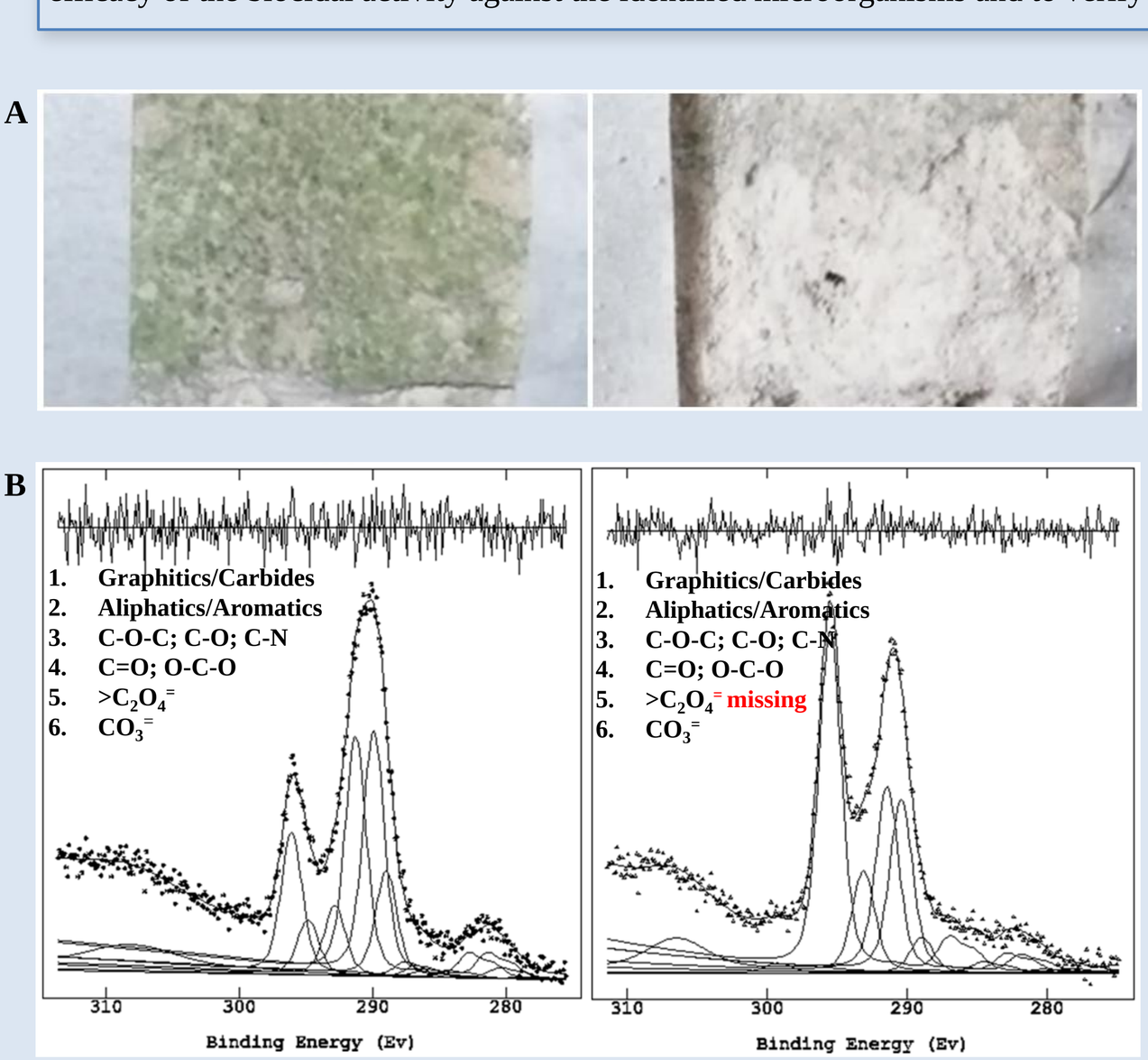

Figure 1 Comparison of calcarenite stones pre (left) and post (right)- GAs treatments:
A) Photographs of the hypogeal sampling areas
B) XPS analysis: curve- fitted resolved C1s regions and peak assignments

Results

Preliminary identification of the fungal species present on the deteriorated surface of the hypogeum structure of the stone church San Pietro Barisano, based on a single locus of the ribosomal RNA, (Internal Transcribed Spacer-ITS), using primers ITS5+ITS4, reported the presence of few fungal genera such as *Penicillum*, *Botryotrichum*, *Phialophora* and *Cladosporium*.
Further molecular identification and characterization at species level based on multilocus molecular analyses (MLA) is in progress.
A photograph of one sampling area, before and after 4-weeks of GA application, is reported in Figure 1A. The powders gently collected by the pre- and post-treated surfaces and properly preserved were analyzed by XPS [**6**]. The carbon 1s details of Figure 1B show that, after four weeks, there is a decrease in the organic component associated with the biofilm activity, the oxalate-like (peak 5) disappearance and an increase in the carbonate component associated to the underneath calcarenite stone.

Conclusions

Fungi belonging to the fungal genera identified in this study, namely *Penicillum*, *Botryotrichum*, *Phialophora* and *Cladosporium* are know to be involved in biodeterioration of historical and cultural stone assets and can also cause health problems to both visitors and guardians. Therefore, their presence on the stone church of San Pietro Barisano (part of the UNESCO heritage) located in Matera (southern Italy) and specifically the hypogeum of the structure is very important in order to prevent biodegradation of the stone asset and protect humans health.
The research performed has allowed to associate the chemical composition of hypogeal surfaces with the bioactivity of the microrganisms identified by biological analysis. The biocidal activity of GAs is evidenced by the results shown in Figure 1. Work still in progress for laboratory test foresees the use of GAs supported in PVA- hydrogels, showing unaltered activity and secure advantage for the application of the proposed method.

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