

# **Salt-weathering and pink biofilms: a widespread problem to be solved**

## **Pretty in pink?**

### **Introduction**

A phenomenon directly related to climate change is the increase in salt-crystallisation cycles on architectural surfaces, due to alternating periods of dry and torrential rains in recent times (Benavente et al., 2008, pp. 277–286). Salt damage architectural surfaces in historic buildings through physico-chemical processes, but it also creates an environmental niche for salt-loving (halophilic) microorganisms. These microorganisms colonise saline environments and produce additional surface alterations through biological exudates and pigment production, resulting in colourful pinkish-red biofilms. These effects threaten our cultural heritage and require innovative conservation strategies, accompanied by a long-term monitoring of their effectiveness. The aim of this study was to identify the structure, function and adaptive strategies of the microbial communities comprising the pink biofilms associated with salt-weathered surfaces. In addition, the effectiveness of a sustainable desalination treatment with different mineral poultices has been tested not only to mitigate the salt damage on the treated surfaces, but also to reduce the associated pink biofilms.

### **Material / Data**

Tests has been addressed under natural conditions in historical buildings displaying high salt loads, and under simulated conditions with mock-ups in a climate chamber.

### **Methodology**

Complementary strategies have been applied, including both culture-dependent and -independent microbiological methods. Selected microbial strains have been used to inoculate mock-ups exposed to simulated climatic conditions similar to those of historic buildings. Furthermore, molecular strategy has been used to analyse the whole genomes of the selected strains and to identify the structure and function of non-culturable members of the microbial community, including sequencing of 16S rRNA long-amplicons and, for the first time, transcriptomic analyses.

Scanning Electron Microscopy (SEM), 3D microscopy and Raman spectroscopy were used to study microbial biofilms and pigments.

Finally, treatments to mitigate salt-weathering were tested on the walls of the two selected buildings using newly designed mineral poultice formulations for long-term application (up to 12 months). During the period of application, pieces of the poultices were taken to the laboratory and used to evaluate the possible co-extraction of salt and biofilms.

### **Results & Conclusions**

The culture-dependent approach leads to the cultivation of salt-adapted species (halotolerant /halophilic) belonging to the kingdom Bacteria and Archaea. Selected strains were subjected to whole genome sequencing using the Nanopore sequencing platform. In addition, these strains were used

to create an "artificial" microbial community to study their functions and potential degradative capacities under simulated conditions when inoculated in mock-ups and incubated in a climatic chamber. The culture-independent approach through full-length 16S rRNA analysis revealed microbial communities selectively adapted to different salt compositions and concentrations. The results indicate that the chemical composition of salts is the main factor affecting the diversity of colonising communities. The presence/absence of certain ions may also produce a shift in the relative composition of the communities established in these hypersaline environments. Preliminary results of the transcriptomic study revealed the versatile strategies used by halotolerant/halophilic microorganisms in relation to osmoregulation, pigment formation and stress resistance mechanisms under extreme conditions.

Complementary multi-analytical analyses helped to understand the extent and nature of the coloured biofilms. SEM analysis showed the intercalated structure of the biofilms in the salt crystals on the wall surfaces and 3D microscopy analysis allowed the same phenomenon to be observed in the artificial communities inoculated in the mock-ups under simulated conditions in the climatic chamber. The results obtained from Raman spectroscopy identified the pink pigments spread on the surface of the walls as bacterial and/or archaeal ruberin (derived from beta-carotene).

Finally, the different mineral poultices tested showed divergent efficacy in both salinity and biofilm reduction depending on the salt load of the different buildings and their chemical composition.

## **Discussion**

This study gives insight into the structure and function of biofilms associated with salt crystallisations, as well as the interconnected strategies of adaptation to different salt concentrations/compositions of biofilm-forming microorganisms. This has enabled the design of new strategies for the long-term preservation of historic surfaces under salt stress.

## **References**

Benavente, D., Brimblecombe, P. and Grossi, C. M. (2008). Salt weathering and climate change. In Colombini, M.P. and Tassi, L. (eds.), *Trends in Analytical, Environmental and Cultural Heritage Chemistry*. TSN Trivandrum, pp. 277–286.