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Analysis of dark crusts on the church of Nossa Senhora do Carmo in Rio de Janeiro, Brazil, using chemical, microscope and metabarcoding microbial identification techniques

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ABSTRACT

The dark crust on the surface of the Nossa Senhora do Carmo church, in the centre of Rio de Janeiro, was shown to contain high salt levels and neogypsum above the original granite. Delamination and scaling were macroscopically obvious. Next generation DNA sequencing techniques were used for the first time to determine the total microbial populations that could be involved in surface deterioration of historic stone buildings in a tropical climate. High levels of halophilic and stress-resistant bacteria and fungi were identified, able to grow at high temperatures, salt and UV levels, and in the presence of low moisture and toxic fossil fuel emissions. Many produced pigments, adding to the discoloration of the stone surface. The most abundant bacterial group was the Actinobacteria, which can burrow into the compromised rock and produce organic acids that further degrade the substrate. Geodermatophilaceae and Micrococcaceae were the major bacterial families. The majority of the fungi identified were dimorphic and present on the façade in their yeast forms, with few filamentous fungi being seen in the scanning electron microscope. Basidiomycetes were the most common group and the Tremellomycetes the most abundant class. Low numbers of phototroph operational taxonomic units, mainly of Chloroflexi, were detected by the MiSeq sequencing method. Initial chemical alterations of the granite surface, together with the climatic conditions in the city of Rio de Janeiro, determined the surface-colonizing microflora. Salt content appeared to be a major driving factor in community structure. This microbial population participated strongly in crust, formation. The final results of biofilm formation on the stone are surface discoloration, induration and degradation of the façade.

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1. Introduction

The granite church of Nossa Senhora do Monte do Carmo is situated on the XV[th] November Square in the centre of Rio de Janeiro, Brazil, within walking distance of Guanabara Bay. It was built, principally of medium grained, garnet-rich granite, in the 16th century, but the present façade was completed in 1822. Like other historic buildings in this area, long exposure to the hot, humid environment and to recent emissions from the intense road traffic in the area have led to the formation of dark crusts, sometimes called patina, on its surface. Such crusts are often present on the exterior walls of historic buildings worldwide. The analysis of particulate matter in patinas on Granada Cathedral in Spain has shown evidence of emissions from leaded-gasoline and sulfur-containing diesel (Rodriguez-Navarro and Sebastian, 1996). The induration (hardening) and darkening of the surface resulting from these depositions leads to fragilization, differential heating and eventual delamination of the stone surface (Scheerer et al., 2009, and references therein).

Various researchers from the Northern hemisphere reported that so-called “black crusts” contain mainly gypsum (e.g., Turtura et al., 2000; Grossi et al., 2003); however, more recently, Gaylarde et al. (2007) demonstrated that thin black crusts on historic buildings in the Mexican town of Campeche were composed

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principally of dark brown filamentous cyanobacteria, with no urban chemical pollutants detected at high levels. These microorganisms produced a hard black coating, which eventually flaked off. These accretions would not correspond to the definition of "black crust" as suggested by ICOMOS in their "Illustrated glossary on stone deterioration patterns" (undated, available at http://www.icomos.org/publications/monuments_and_sites/15/pdf/Monuments_and_Sites_15_ISCS_Glossary_Stone.pdf).

Microbial surface assemblages (biofilms) such as those discussed above, have also been implicated in the solubilisation of iron and manganese from within rocks, with subsequent re-deposition of these metals on the rock surface and formation of dark films (Barrionuevo et al., 2016). Thus, microorganisms may be heavily involved in the deterioration and degradation of stone buildings. While it is accepted that cyanobacteria, algae and fungi can contribute significantly to the overall deterioration of stone, as well as many other building materials used in architecture, the role that non-cyanobacteria bacterial groups play in this process has only rarely been studied in depth (Tayler and May 1991, 2000; Ortega-Morales et al., 2004).

Although darkening, blistering and scaling on the façade of Nossa Senhora do Carmo church were first described by Smith and Magee (1990), to our knowledge no detailed chemical and microbiological analyses have been carried out on this building. Early studies on microbial growth on built structures, mainly in Europe, used cultivation-based techniques, microscopy, or *in vitro* culturing coupled with molecular PCR-methods to identify the microorganisms present on stone constructions (McNamara et al., 2006; Gorbushina, 2007; Cuzman et al., 2010; Gleeson et al., 2010). Most of the reported organisms were the fast growing, easily cultured species. Modern molecular techniques have only recently, and rarely, been applied to interrogate the microbiome on heritage building materials (Cutler and Viles, 2010; Gutarowska et al., 2015; Cutler et al., 2015), and these typically reveal much higher microbial diversity than that obtained from environmental enrichments. Methods of PCR and DGGE have been employed to analyse the bacteria, phototrophs and fungi on the buildings and in the air of a courtyard in the University of Milan, Italy (Polo et al., 2012). However, only 10 bacterial genera, 6 fungal genera, 2 algae and 1 cyanobacterium were detected in that study. The first reported use of Next Generation Sequencing (NGS) techniques for analysis of DNA recovered from microbial populations thriving on historic structural materials was published by Gutarowska et al. (2015); however, they did not investigate stone buildings. Recently, the phototroph and fungal, but not bacterial, populations on historic granite buildings in Santiago de Compostela, Spain, were analysed using NGS techniques (Vázquez-Nion et al., 2016). The expanding portfolio of rapid, high throughput molecular microbial ecology methods allows the characterization of microbial communities more rapidly and more efficiently than the cultivation approach. However, one single method is not sufficient to properly analyse the diversity of colonizing microorganisms. Brandes et al. (2015) pointed out that many cyanobacterial morphotypes have never been subjected to molecular analysis and hence their sequences are missing from the gene banks. Herein, we report the results of NGS, as well as chemical and microscopy analyses, to investigate the interactions between microorganisms and the stone surface of dimensional granite, as exemplified by the façade of Nossa Senhora do Carmo church.

2. Materials and methods

2.1. Sites and sampling

Samples were obtained from the front façade of the church of Nossa Senhora do Monte do Carmo, situated on Praca XV de Novembro, central Rio de Janeiro, RJ, Brazil. Specimens were taken randomly at a height of 1–2 m, using the adhesive tape technique (Shirakawa et al., 2002) and by collecting samples of contour scales composed of surface crust and underlying granite (Fig. 1) from areas of around 1 m × 50 cm. The latter sampling procedure ensured that the interface between the two different materials, which may harbor particular microorganisms (Saiz-Jimenez and Laiz, 2000), was preserved.

2.2. Chemical analyses

Water-soluble salts were extracted from representative portions of fragmented crust samples by shaking the material in deionised water for two hours at 23 °C. After overnight soaking the solutions were centrifuged at 3500 × g and supernatants filtered through a 0.2 μm membrane (Millipore). The cations Ca, Mg, Na, K, Fe, Cu, Fe, Mn, Ni, Pb and Zn were measured using a Perkin Elmer Model 3100 atomic absorption spectrometer. Water-soluble anions F, Cl, NO_3, PO_4 and SO_4 were analysed using a Dionex Model DX 500 ion chromatograph with an Ion-Pac AG4A-SC (4 mm) guard column and AS4A-Sc (4 m) anion exchange column. The injection loop was 25 μ, eluant consisted of 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ at a flow rate of 2 mL per minute and a conductivity detector with an Anion Self-Regenerating Suppresser (ASRSTM) was employed. Detection limit for all anions was 1 ppm. The pH of stone fragments was determined in deionised water using an Orion Model 410A meter. Six samples were analysed in total.

2.3. Microbiological investigations

Two adhesive tape samples were placed on a glass microscope slide, rehydrated with sterile deionised water and examined under the light microscope (Leica) with x 10 to x 40 objectives. Two or three fragments of crust samples were imaged using field emission scanning electron microscopy (FE-SEM), characterized by energy dispersive x-ray (EDX) analysis and processed using DNA analytical techniques. Two samples were also examined by light microscopy, after grinding and rehydration. The 16S rRNA region of extracted DNA was analysed employing Next Generation Sequencing (NGS) MiSeq.

2.3.1. FE-SEM/EDX

Selected, representative specimens were sputter-coated with gold-palladium to obtain approximately 5 nm thickness of Au-Pd deposit. FE-SEM images of the coupons were acquired using the Zeiss Neon 40 EsB high resolution FE-SEM equipped with EDX. The accelerating voltages were 5 and 20 kV, using the Schottky emitter (Gemini lens SEM column) electron source. For EDX analysis the Oxford x-ray detector was employed with proprietary software (Lenhart et al., 2014). Images were also obtained after coating samples with iridium and observing with a Zeiss SUPRA 55VP Ultra-High Resolution FE-SEM.

2.3.2. DNA extraction

Approximately 6 cm^2 (average 2.1 g) of each of 2 selected stone samples (C1 and C2) was ground with a sterile pestle and mortar and weighed. Aliquots were then subjected to DNA extraction, using the PowerSoil DNA isolation kit (MO-BIO Laboratories, Carlsbad, CA, USA), which includes a bead-beating step. After the final spin of the PowerSoil procedure, DNA was extracted using the Maxwell 16 Tissue LEV Total RNA Purification kit (Promega WI, USA), following the manufacturer's instructions. This mixed procedure has been shown, in our laboratory, to be extremely efficient for DNA extraction from concrete, stone and corroding carbon steel. Finally, the DNA concentration was determined with the Qubit® dsDNA BR

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