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Diversity of archaeal and bacterial communities on exfoliated sandstone from Portchester Castle (UK)

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ABSTRACT

In this study exfoliated sandstone samples from Portchester Castle were investigated using scanning electron microscopy (SEM) and energy dispersion X-ray (EDX) analyses to observe stone surface colonisation, geomorphological structure and to assess damage. Archaeal and bacterial diversity were assessed using cultivation-dependent and cultivation-independent methods. SEM analysis showed that sandstone had high levels of stone decay. There was considerable weathering of the minerals associated with biofilms containing microbes with various cellular morphologies. Microorganisms were especially prevalent in pores, cavities and in the heavily decayed parts of the minerals, and some etching was seen. EDX analyses indicated microbes were associated with the sheet structures of aluminium-containing phyllosilicate minerals, most likely glauconite. Microbial colonisation was preferentially concentrated within specific sheets of the mineral structure. Isolation studies revealed the presence of *Bacillus* and *Arthrobacter* that appeared to be well adapted to "extreme" environments, specifically these isolates were tolerant to high salt, high UV and oligotrophic conditions. Cultivation-independent studies using denaturing gradient gel electrophoresis fingerprinting of bacterial and archaeal 16S rRNA gene fragments showed a more complex community. Chloroflexi, Actinobacteria, *Deinococcus*, α- and β-proteobacteria, Cyanobacteria and Bacteroidetes and halophilic *Archaea* from the family Halobacteriaceae, were the predominant types of *Bacteria* and *Archaea* detected respectively.

1. Introduction

Cultural heritage artefacts are affected by chemical and physical processes that can modify their structure and composition. Microorganisms associated with such artefacts are known to cause deterioration in organic material as well as inorganic materials such as stonework. A wide variety of microorganisms, especially bacteria and fungi, have been found on rocks and the stonework of historic monuments and buildings (McNamara and Mitchell, 2005; Crispim and Gaylarde, 2005; Gorbushina, 2007; Ranalli et al. 2009; Scheerer et al. 2009; May 2010; Cappitelli et al. 2012).

Biodeterioration processes result from a complex interaction of microorganisms with the surface material, together with environmental and chemical factors that influence the aggressiveness of the process. Stone pore spaces and cavities protect microbial communities from solar radiation and desiccation, as well as providing mineral nutrients, moisture and a growth surface. In addition, when a biofilm is present, its extracellular polysaccharides provide further protection against a variety of environmental stresses, such as UV radiation, changes in pH, osmotic shock and desiccation and reduce the penetration of antimicrobial agents (Alakomi et al. 2006).

An appropriate assessment of stone biodeterioration and weathering effects requires a combination of microbiological, surface analysis and material characterization techniques. This typically involves the identification of the major types of

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microorganisms present, microscopic observations of the biofilm/material interface, elemental and mineral analysis of the damaged material and assessment of the morphology of the decay. Among the different methods for material characterization and surface analysis, scanning electron microscopy (SEM) and energy dispersion X-ray analysis (EDX), are two of the most useful techniques for assessing both biotic and abiotic aspects of weathering and biodeterioration on cultural heritage materials.

SEM allows surface characterization at a high resolution, permitting visualisation of the microorganisms involved, their chemical and mechanical effects and the extent of biodeterioration. In previous studies on cultural heritage sites, SEM has revealed the microbial community as a complex interacting system, present in epilithic and endolithic zones that can be detrimental to monument stone. Effects on mineral species inside fissures and possible chemical changes are, in various ways, determined by the nature of the colonizing microbial populations (Herrera and Videla, 2009; de los Rios and Ascaso, 2005; Polo et al. 2010).

From a biological point of view, a deeper understanding of the nature of the microbial community involved in these biodeterioration processes has improved in the last few decades with the application of molecular biological methods. Such techniques complement and expand the information on the resident microbes, which previously had been gathered by conventional microbial identification methods. Some studies have focused on the analysis of the structure of the colonising microbial communities, on the optimisation of molecular techniques in the field and on the detection of specific biodeteriogenic agents in the alterations. Other studies revealed the presence of novel microorganisms, which had never been detected in these environments before. Many investigations have been performed on objects in indoor environments such as wall paintings, frescoes and cave paintings; others on outdoor stoneworks of artistic and historical significance (Daffonchio et al. 2000; Urzì et al. 2001; Alonso-Vega et al. 2011; Piñar et al. 2001; Portillo et al. 2009; Saiz-Jimenez et al. 2011; Gurtner et al. 2000; Ripka et al. 2006; Abdulla et al. 2008; Giacomucci et al. 2011). There is still a clear need for in-depth investigation of the microbial species involved, in order to evaluate their role in biodeterioration. The need to identify the members of the microbial community that cause biodeterioration and to understand their specific roles is crucial, in order to effectively protect monuments through appropriate conservation plans and to maintain a long-term conservation strategy of our historic and artistic patrimony (Ranalli et al. 2009; May 2010; Saiz-Jimenez et al. 2011).

The overall aim of the work presented in this paper was to study the diversity and distribution of the microbial community associated with sandstone alterations (mainly exfoliations) of outdoor stonework at Portchester Castle near Portsmouth using a combination of microscopy techniques and microbiological and molecular analyses. Of particular interest was the occurrence of those types of organisms that may be adapted to the harsh stone environment with possible resistance to high salt concentration, oligotrophic conditions and radiation.

2. Materials and methods

2.1. Description of study site

Portchester Castle is a medieval castle built on a former Roman fort at Portchester to the east of Fareham, Hampshire, UK. It lies at the head of Portsmouth Harbour (see Fig. 1a). It is uncertain exactly when the castle was built, although it was probably in the late 11th century. Purbeck limestone was used to build fortification walls of the inner keep and green sandstone can be found in its buttresses and decorative door and window arches. Many parts of the castle in sheltered areas have macroscopic signs of biological colonisation, especially lichens and green patinas. The sandstone contains a considerable amount of clay and shows extensive decay. The east fortification wall, which faces the sea, suffers from extensive erosion due to salt and wind action.

South-East England is close to continental Europe and experiences a climate consistent with air masses from both continental Europe and from depressions crossing the Atlantic, bringing cold spells in winter and hot, humid weather in summer. The relatively sheltered nature of the region relative to these Atlantic depressions means, however, that their impact is less than experienced across the western parts of the UK (Wheeler and Mayes, 1997). The south coast of this region has higher sunshine than the UK average, at over 1541 h per year, with relatively low rainfall (mean annual rainfall for Southsea between 1961 and 1990 was 685 mm) and relatively mild temperatures (annual mean temperature for Southampton 1961–1900 of 10.8°C) (Wheeler and Mayes, 1997). Coastal sea breezes at Portsmouth, however, provide an important additional source of salt spray in what is otherwise a relatively mild wind climate (Harrison, 1976).

2.2. Sandstone sampling at portchester castle

Samples of green sandstone were taken from two sampling sites on a window in Richard II's banqueting room within the keep of the castle (Fig. 1b). Sites 1 and 2 (Fig. 1c and d respectively) were to the lower right of the window and both showed extensive exfoliation (Fig. 1e). Three replicate samples (1 A, B, C and 2 A, B, C) were aseptically removed from each site in June 2009 from a surface area of 10 cm^2. The samples were kept at 4°C until they were transported to the University of Warwick (UK) for further processing.

Green sandstone is classified as very fine sand, glauconitic lithic arenite, highly porous stone with a mineral composition of: Quartz (SiO_2) 48%, Calcite ($CaCO_3$) 20%, Glauconite 14%, Limonite 6%, fine grained rock fragments 12% and occasional Muscovite and Felspar (Lewis, 1987). Texture is composed by dominant grain size (1/16–1/18 mm), moderately to well-ordered, and porosity is 32.3%. The high porosity of the sandstone permits relatively easy ingress of fluids and microbes into the matrix of the sandstone whilst the grain size provides a relatively large surface for microbes to colonize within the sandstone. The chemical composition of the sandstone provides a potentially resource-rich environment for microbial growth, particularly with the high concentration of glauconite containing iron and potassium.

2.3. Scanning electron microscopy analyses

After collection, stone samples for SEM were fixed overnight in 4% v/v glutaraldehyde in 0.2 M sodium cacodylate buffer, rinsed in buffer, and then fixed in 1% w/v osmium tetroxide in 0.2 M sodium cacodylate buffer. After rinsing in distilled water, samples were dehydrated in a graded ethanol series (15 min holding time in 20% v/v steps) from 30% to absolute alcohol and then transferred to absolute acetone. After critical point drying in a Polaron Thermocirculator E3500, samples were glued to aluminium stubs and sputter coated with a gold/palladium mixture in a Polaron E5000 diode sputtering system. Samples were examined by SEM with a JEOL-JSM 6060 LV microscope attached to a X-Max EDX system (Oxford Instruments).

2.4. Plate counts and isolation of heterotrophic bacteria

The stone samples (~0.5 g from all three aliquots pooled for the plating assays) were crushed using a sterile mortar and pestle and resuspended in 10 ml phosphate-buffered saline (PBS) amended