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Bacterial diversity on rock surface of the ruined part of a French historic monument: The Chaalis abbey

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ABSTRACT

The aim of the present study was to analyze the epilithic bacterial diversity of the ruins of the Chaalis Abbey, France. To do this, stone samples were collected at the surface of three areas presenting different macroscopic characteristics: a Discolored Area (DCA), a Damaged Area (DMA) and an Undamaged Area (UDMA). The bacterial diversity investigation after DNA extraction and amplified ribosomal DNA restriction analysis targeting the 16S rRNA genes revealed that clone libraries were dominated by *Alphaproteobacteria* and *Actinobacteria*. The main part of recovered phylotypes belonged to genera previously associated with stone colonization and/or biodeterioration. Indeed most abundant *Alphaproteobacteria* phylotypes belonged to the genus *Sphingomonas* and to the species *Bosea thiooxidans*, and most abundant *Actinobacteria* phylotypes belonged to the genera *Rubrobacter* and *Arthrobacter*. Members of *Cyanobacteria*, *Bacteroidetes*, TM7, *Betaproteobacteria*, *Deinococcus*, *Acidobacteria* and *Chloroflexi* were also recovered but to a lesser extent. In conclusion, these results suggest that the ruins of the Chaalis Abbey are covered by bacterial communities mainly composed of *Alphaproteobacteria* and *Actinobacteria* able to colonize and, for some of them, to deteriorate stone.

1. Introduction

The surface of stone monuments exposed to the outside is continually subjected to physicochemical environmental factors that act as weathering agents: wind, temperature, rain, relative humidity, condensation, and air pollution. These environmental factors can more or less alter the rocky substrate in accordance to its mineralogical composition and structure (Miller *et al.*, 2012). Biological factors also impact stone deterioration processes. Biodeterioration is defined as "any undesirable change in a material brought about by vital activities of organisms" (Hueck, 2001). Microorganisms that play a potential role in biodeteriorative processes are autotrophic and heterotrophic bacteria, fungi, algae, lichens and protozoa (Gómez-Alarcón *et al.*, 1995; Tomaselli *et al.*, 2000; Gaylarde and Gaylarde, 2005; Sterflinger, 2010). This consortium of heterogeneous microbial species forms a biofilm where microbial cells are embedded in extracellular polymeric substances (EPS) (Gorbushina, 2007; Di Martino, 2016). The interaction of microorganisms and building stone is complex and has been reviewed extensively (Gaylarde and Morton, 1999; *Griffin et al.*, 1991; Kumar and Kumar, 1999, Mihajlovski *et al.*, 2015; Saiz-Jimenez, 1999; 2001; Sand and Bock, 1991; Scheerer *et al.*, 2009, Steiger *et al.*, 2011, Warscheid and Braams, 2000; Urzì, 2004). Indeed, the stone surface of monuments is a common habitat for a wide range of microorganisms. Microbial communities development occurs at the interface between the stone and the atmosphere, on top of or inside the rock (de los Rios *et al.* 2004). Microorganisms can cause various damages on stone surfaces. The biodeterioration impact of microorganisms can be classified into three categories that may occur separately or simultaneously, namely: (i) biophysical (ii) biochemical and (iii) aesthetic. These different categories have been described and reviewed by several authors (e.g. Allsopp *et al.*, 2004; Gaylarde *et al.*, 2003; Griffin *et al.*, 1991; Kumar and Kumar, 1999, Saiz-Jimenez, 2001; Scheerer *et al.*, 2009, Warscheid and Braams, 2000). Biophysical deteriorations refer to actions that directly affect the material components and its mechanical properties, such as the fungal hyphal growth into the

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mineral support (Gadd, 2007; Hoffland *et al.*, 2004). Biochemical deteriorations can be divided into: assimilatory and dissimilatory processes. Assimilatory process occurs when microorganisms use the mineral support as a source of nutrients and energy. This can results in the modifications of the material properties. In dissimilatory processes, microbial metabolism products can chemically react with the mineral support and thus alter the latter. Finally, even if biophysical and biochemical biodeteriorative processes can lead to aesthetic deterioration, the latter is often associated to the discoloration of the support. These discoloration can be caused by pigments released from, or contained within, the microorganisms and also from the biofilm EPS that facilitate entrapment of airborne particles, aerosols, minerals, and organic compounds and thus increase the dirty appearance of the substrate (Kemmling *et al.*, 2004). Even if the materials properties are not initially affected, as time passes the fouling biofilm impact may exceed the purely aesthetic consideration and may cause physicochemical damages to the support. The concept of bioreceptivity was defined by Guillitte (1995) as "the aptitude of a material to be colonized by one or several groups of living organisms without necessarily undergoing any biodeterioration" or as "the totality of material properties that contribute to the establishment, anchorage and development of fauna and/or flora" (Guillitte, 1995). Thus, the intrinsic properties of the stone material itself, i.e., surface roughness, porosity and chemical composition also influence microbial colonization and deterioration processes (Miller *et al.*, 2012). In addition, as the intrinsic characteristics of the stone materials change over time, bioreceptivity is not considered as a static property and several types of bioreceptivity exit (Guillitte, 1995). Thus, the state of conservation of building materials can also affect its bioreceptivity (Ortega-Calvo *et al.*, 1995) as well as the accumulation of exogenous deposits such as soil, dust or organic particles (Guillitte, 1995). Carbonate stones such as marble and limestones are particularly sensitive to deterioration by physical and chemical weathering and biodeterioration associated to the presence of macro-and microorganisms (Lamenti *et al.*, 2000; Saiz-Jimenez, 2001). As a large number of the most important cultural heritage structures are built with carbonate stones, their conservation for long period of times is at risk (Gauri and Bandyopadhyay, 1999). Finally, the colonization of the outdoor surface of monuments is heterogeneous depending on the weathering level of the studied area (de los Ríos et al., 2009; Qi-Wang *et al.*, 2011). This suggests that these variable weathering levels may be due to different aggressive colonizers or that they may lead to the colonization by various kinds of microorganisms.

The purpose of this study was to analyze the diversity of bacteria living at the surface of the ruins of the royal abbey of Chaalis north of Paris, France. In order to get a global characterization of the epilithic bacterial diversity present on the outdoor face-wall of the monument, stone samples were collected from areas presenting various degrees of deterioration (Fig. 1 and Fig. 2).

2. Material and methods

2.1. Sampling site and samples

The royal abbey of Chaalis is located in the north of Paris near Senlis. The domain, classified as historic monument since September 9, 1965, contains the ruins of the old abbey, the old abbey chapel and its frescoes of the Renaissance and a park with a rose garden (Fig. 1). Monuments of the abbey are built with Lutetian limestone, a material widely used in the construction of monuments in France, which has characteristics of porosity and roughness conducive to bacterial growth (Vázquez *et al.*, 2015). In order to get a global characterization of the epilithic bacterial diversity present on the outdoor face-wall of this monument, stone samples of the ruins of the old abbey were collected from three areas presenting different degrees of deterioration in March 2012. These areas are presented in Fig. 2. The Discolored area (DCA, Fig. 2B) designates a grey/black area subjected to water run-off and presenting mosses and yellow lichens. The Damaged Area (DMA, Fig. 2C) designates an area not subjected to water run-off where a loss of limestone is observed. Finally the visually Undamaged Area (UDMA, Fig. 2D) is an area not subjected to water run-off presenting no discoloration and no erosion of the stone support. A surface of 3–5 cm^2 was aseptically scrapped off from the surface of each area and stored at −20 °C until use.

2.2. DNA extraction

Nucleic acids were extracted from stone samples using a protocol adapted from Zhou *et al.* (1996). Around 500 mg of the surface of stone samples were scraped off using sterile scalpels and suspended in 1 mL of lyses buffer (Zhou *et al.*, 1996). Locations presenting lichens were avoided. Following this, 200 μg of proteinase K was added and the tube incubated at 37 °C for 30 min, with shaking at 500 rpm (Thermomixer Eppendorf). Then 15 μL of 20% (w/v) SDS was added and incubation continued at 65 °C for a further 2 h. After centrifugation at 6000*g* at room temperature for 10 min, the supernatant fluid was collected.

The pellet was extracted two more times with 250 μL of lyses buffer supplemented with 5 μL of 20% SDS, followed by incubation at 65 °C for 10 min. For each sample, the three supernatants fluids were pooled and nucleic acids extracted with chloroform/isoamyl alcohol (24:1, v/v) and precipitated with isopropanol. After centrifugation at 16 000*g* for 20 min at room temperature, the nucleic acid pellets were air dried and re-suspended in 100 μL TE. After adjustment of the salt concentration of nucleic acids solutions to allow their binding to the silica membrane, nucleic acids were purified using the « Power Biofilm™ DNA Isolation Kit » (MoBio) following manufacturers instruction. Nucleic acid extracts were then stored at −20 °C until use.

2.3. Amplification and cloning of 16S rRNA genes

Bacterial 16S rRNA genes were amplified by PCR using the forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and the reverse primer 1492R (5′-TACGGYTACCTTGTTACGACT-3′). Fourteen bases were added to the forward primer for oriented cloning in the pSTC1.3 vector (StabyCloning kit, Eurogentec, Belgium), leading to primer 27F_pSTC (5′–*CCTTCgCCgACTgA*AGAGTTTGATCMTGGCTCAG-3′). Reactions were performed in a total volume of 25 μL comprising 20 ng of DNA, 100 μM of each dNTP, 0.4 μM of each primer, 1X PCR buffer and 1 U of Accusure DNA Polymerase (Bioline, France). The following conditions were used for DNA amplification: 10 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C and 2min20 s at 68 °C, and a final extension at 68 °C for 5min. Resulting amplicons of the expected size (around 1500 pb) were extracted from an agarose gel after electrophoresis using the Nucleospin Gel and PCR clean up kit (Macherey Nagel, France) and orientally inserted in the pSTC1.3 vector of the StabyCloning kit (Eurogentec, Belgium) following the manufacturers' instructions. Transformed electrocompetent *Escherichia coli* CYS21 cells (provided with the kit) were then spread-plated onto LB agar containing 50 μg/mL ampicillin and incubated overnight at 37 °C.

2.4. RFLP screening of libraries

PCR amplifications of the inserts using primers which target the pSTC1.3 vector: pSTC-F (5′-AATGCAGCGCGTTAGAA-3′) and pSTC-R