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Microscopic, chemical, and molecular-biological investigation of the decayed medieval stained window glasses of two Catalonian churches

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ABSTRACT

We investigated the decayed historical church window glasses of two Catalonian churches, both under Mediterranean climate. Glass surfaces were studied by scanning electron microscopy (SEM), energy dispersive spectrometry (EDS), and X-ray diffraction (XRD). Their chemical composition was determined by wavelength-dispersive spectrometry (WDS) microprobe analysis. The biodiversity was investigated by molecular methods: DNA extraction from glass, amplification by PCR targeting the16S rRNA and ITS regions, and fingerprint analyses by denaturing gradient gel electrophoresis (DGGE). Clone libraries containing either PCR fragments of the bacterial 16S rDNA or the fungal ITS regions were screened by DGGE. Clone inserts were sequenced and compared with the EMBL database. Similarity values ranged from 89 to 100% to known bacteria and fungi. Biological activity in both sites was evidenced in the form of orange patinas, bio-pitting, and mineral precipitation. Analyses revealed complex bacterial communities consisting of members of the phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. Fungi showed less diversity than bacteria, and species of the genera *Cladosporium* and *Phoma* were dominant. The detected Actinobacteria and fungi may be responsible for the observed bio-pitting phenomenon. Moreover, some of the detected bacteria are known for their mineral precipitation capabilities. Sequence results also showed similarities with bacteria commonly found on deteriorated stone monuments, supporting the idea that medieval stained glass biodeterioration in the Mediterranean area shows a pattern comparable to that on stone.

1. Introduction

Medieval stained glass windows are part of our cultural heritage, but due to their permanent exposure to environmental conditions they have been damaged over centuries. To protect and conserve this valuable material, it is necessary to understand the long-term environmental corrosion processes on glass. Stained glass is made up of several components: network formers, stabilizers, modifiers, and coloring elements (Römich, 1999). The main network formers used in medieval stained glass were silica and phosphorus. In addition, several metals, such as Cu, Co, and Mn, were used to color the glass (Bamford, 1977; Newton and Davison, 1989). Nevertheless, if we consider the main chemical elements, historic glass can be classified into two types: K-rich and Na-rich glass (Newton and Fuchs, 1988; Brill, 1999). Most Central European medieval stained glass produced between the 12th and 15th centuries was K-rich in composition. However, the coeval European Mediterranean glass shows the continuation of a Roman-like Na-rich glassmaking tradition. A common feature of medieval stained glass is the presence of corrosion, patina development, and mineral crust growth over the glass, which has caused serious damage to many Central European stained glass windows. For this reason, studies on glass decay have become important in several countries. Most of these studies consider that glass corrosion and decay are related mainly to a physicochemical process (Newton and Davison, 1989; Schreiner, 1991). Nevertheless, since the beginning of the 20th century there has been evidence of biological induction in stained glass decay (Krumbein et al., 1995). Other important factors that enhance corrosion are environmental pollution (i.e., CO_2 and SO_x in urban areas, where most stained glass windows are located) and, in the case of biodeterioration, the presence of organic carbon on the glass.

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The result of glass decay is a sharp decrease of the flux and network modifiers on the surface and contiguous mass of glass (leaching). This leads to the genesis of a gel surface (or planar volume) of the glass depleted in practically all glass components except network formers (Newton and Davison, 1989; Sterpenich and Libourel, 2001; Garcia-Valles et al., 2003). The leached elements may combine with other (i.e., atmospheric) components to form complex salts. The most soluble salts are removed by moisture and rain, but the others remain on the glass surface as mineral products forming patinas and crusts, as sulfates (gypsum and syngenite), calcite, Ca-oxalates, etc. In K–Ca-rich medieval glasses, when the surface pH might reach a level greater than 9, advanced corrosion of the outer level of silica-rich glass occurs (Garcia-Valles et al., 2003).

As mentioned above, nowadays biological corrosion of glass is a well-known phenomenon. Glass biodeterioration is the result of metabolic activities of complex microbial communities composed mainly of fungi (Nagamuttu, 1967; Kaiser et al., 1996; Schabereiter-Gurtner et al., 2001b), bacteria (Rölleke et al., 1999; Marvasi et al., 2009), and lichens (Mellor, 1924). The organic residues present on historical glass, as dead microbial material, metabolites of autotrophic bacteria, animal faeces, and dust deposits, promote the microbial growth. The role of microorganisms in glass decay includes both chemical and mechanical destruction of glass. The mycelia of filamentous fungi and Actinobacteria initiate both a mechanical destruction and the creation of a leaching environment by the adsorption of water, enhancing the chemical destruction of glasses. Furthermore, the production of metabolic products, as organic and inorganic acids, can lead to pH changes, redox-reactions leaching and chelation of special glass components. In summary, microbial growth on glass surfaces produces several types of damage, such as bio-pitting corrosion, cracks, and patina formation (Krumbein et al., 1991; Drewello and Weissmann, 1997).

Investigations of microbial colonization of historical glass have so far been based on culture-dependent methods (Krumbein et al., 1991; Drewello and Weissmann, 1997), with only the exceptions using culture-independent techniques (Rölleke et al., 1999; Schabereiter-Gurtner et al., 2001b; Carmona et al., 2006). In determining the appropriate measures to take against microbial growth on historical glass, it is important to get an overview of the inhabiting microbial populations. However, the first problem found when working with samples taken from cultural assets is the small quantity of sample material available, which is in most cases not sufficient for reliable cultivation assays. Furthermore, microbes are usually members of complex microbial communities and depend on special nutrients; therefore only a minority of them can be cultivated under conventional laboratory conditions. By contrast, cultivation-independent methods enable the detection of slowly growing, fastidious, or uncultivable microorganisms, allowing a more complete picture of the inhabiting microbial communities than do traditional cultivation techniques (Schabereiter-Gurtner et al., 2001a).

The objective of this study was the chemical and biological characterization of the stained window glasses showing signs of biodeterioration of two Catalonian churches. To this end, glass surfaces were investigated using scanning electron microscopy (SEM), energy dispersive spectrometry (EDS), and X-ray diffraction (XRD). In addition, the chemical glass composition was investigated using wavelength-dispersive spectrometry (WDS) microprobe analysis.

The biodiversity of the micro-biota associated with the observed decay was investigated by the following molecular methods: DNA extraction from glass samples, amplification by PCR targeting the16S rRNA and ITS regions, and DNA fingerprint analyses by denaturing gradient gel electrophoresis (DGGE). In parallel clone libraries containing either PCR fragments of the 16S rDNA or the ITS regions were screened by DGGE and selected clone inserts were sequenced and compared with the EMBL database.

2. Materials and methods

2.1. Sampling

Glass samples were obtained from different restoration and conservation projects in two Mediterranean coastal cities of northeastern Spain, Tarragona and Barcelona. This area has a Mediterranean climate with rainfalls mainly concentrated in spring and fall (around 580 mm yr^{-1}) and is characterized by moderate weather, warm summers (21–30 °C), and mild sunny winters (6–14 °C). Both buildings are located ca. 150 m from the marine shoreline.

The rosette glasses of the transept from the Cathedral of Tarragona (Fig. 1) date back to the beginning of the 14th century. They show evidence of damage and repair after the War of Independence (1808–1812) as well. All stained glass windows show original black-fired draws (grisailles), but our present study is only concerned with the composition and corrosion of the main pieces of glass.

The church of Santa Maria del Mar in Barcelona (Fig. 2) is a medieval building erected in the 14th century but the large rosette on the main façade was destroyed by the 1428 Pyrenees earthquake and the stained glass window was rebuilt during the 15th century. Over the centuries, damage and repairs have led to a mixture of old glass panels and new ones, with the most important modifications probably dating from the Spanish War of Independence and the Spanish Civil War (1936–1939) (Ainaud de Lasarte et al., 1985).

All samples were obtained during restoration works and therefore consist of small pieces of broken glass (in general smaller than 0.5 g) that have no possibility of being remounted in the panels.

2.2. Analytical methods

The samples were first observed through a stereomicroscope to obtain morphological information, determine the structure and texture of the surface, determine the conservation state of grisaille, and observe the weathering products (patinas, crusts, pitting, loss of material, etc.). This was done to select the most suitable areas of the surface glass to be scraped with a diamond grindstone and to concentrate the neo-formed phase powder, which was mineralogically identified using a SIEMENS D-500 X-ray diffractometer. Diffraction patterns in the range 4–70° 2θ were obtained with a 0.05° 2θ step scan and 5 s counting time, using Cu Kα radiation, tube conditions of 40 kV and 28 mA, and a graphite monochromator.

The glass samples were cut into two pieces. One was used to study the fresh fracture, including the glass and neo-formed surface, by scanning electron microscopy (SEM). The instruments used were a JEOL J3M-840 and a Leica 360, both served by a LINK Microanalysis energy dispersive spectrometry EDS system, including an energy-dispersive X-ray spectroscopy detector facility (LINKAN 10000 EDS). The other section, perpendicular to the surface, was set in an epoxy resin block, and then by SEM-EDS. Scanning electron microscopy was used to determine the structural changes in the surface, to evaluate the rate of corrosion within the glass, and to determine the composition.

The chemical composition of the glass was obtained using wavelength-dispersive spectrometry (WDS) microprobe analysis