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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

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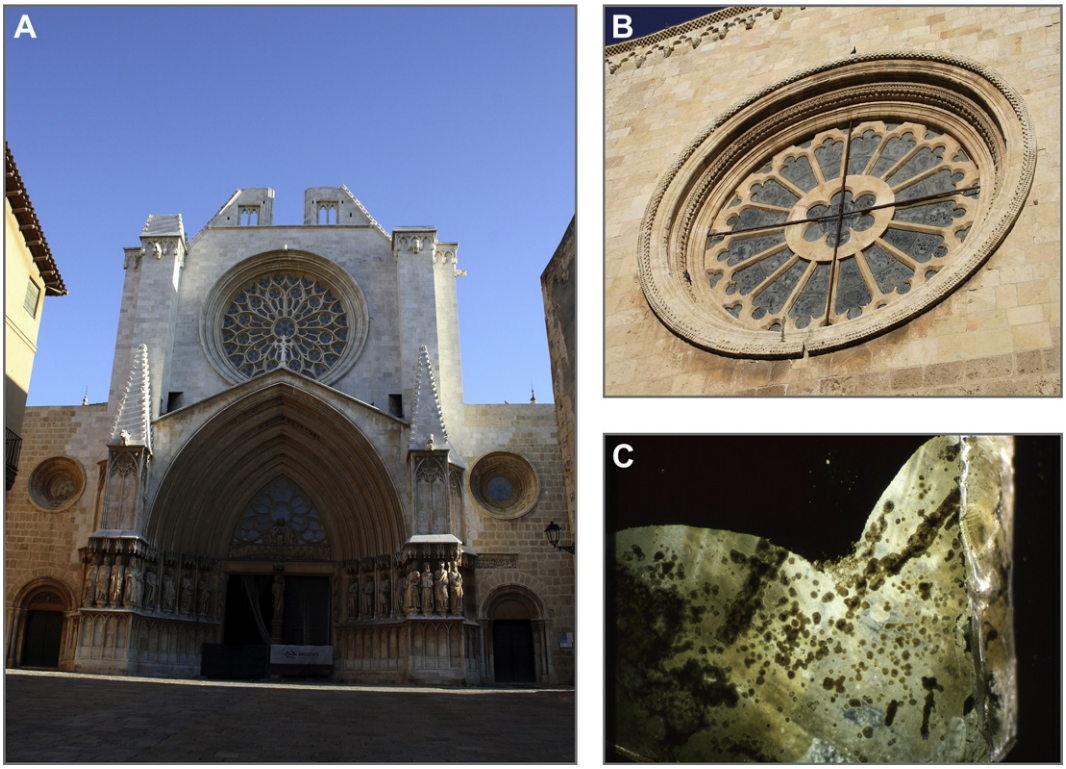

Fig. 1. Cathedral of Tarragona. 1A: Detail of the façade. 1B: Detail of the rosette glasses of the transept from the Cathedral of Tarragona. 1C: Detail of a glass piece with deterioration signs looking like *Cladosporium* attack.

(CAMECA Camebax SX-50). Different natural and synthetic silicates and oxides of certified composition were used as standards (P&H Developments and Agar Scientific commercial standard blocks). The analyzing crystals were provided by CAMECA (LIF, TAP, and PET; and PC0 for instrumental determination of oxygen). EPMA was used for the quantitative chemical characterization of the glass. This was achieved by random point microanalysis of the fresh glassy mesostase (in general $n = 15$ over each fragment). Outside of the leached surfaces glasses are very homogeneous and analytical differences are within the range of (or much less than) expected

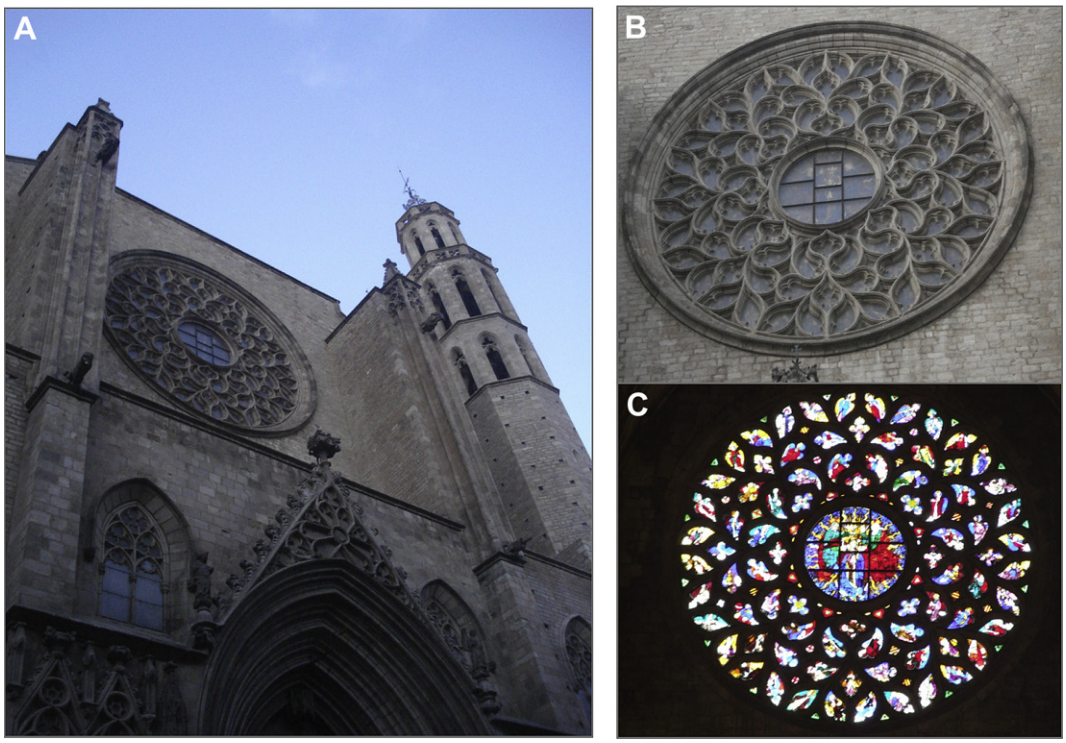

Fig. 2. Church of Santa Maria del Mar, Barcelona. 2A: Detail of the façade. 2B: Detail of the outer rosette glasses. 2C: Detail of the inner rosette glasses.

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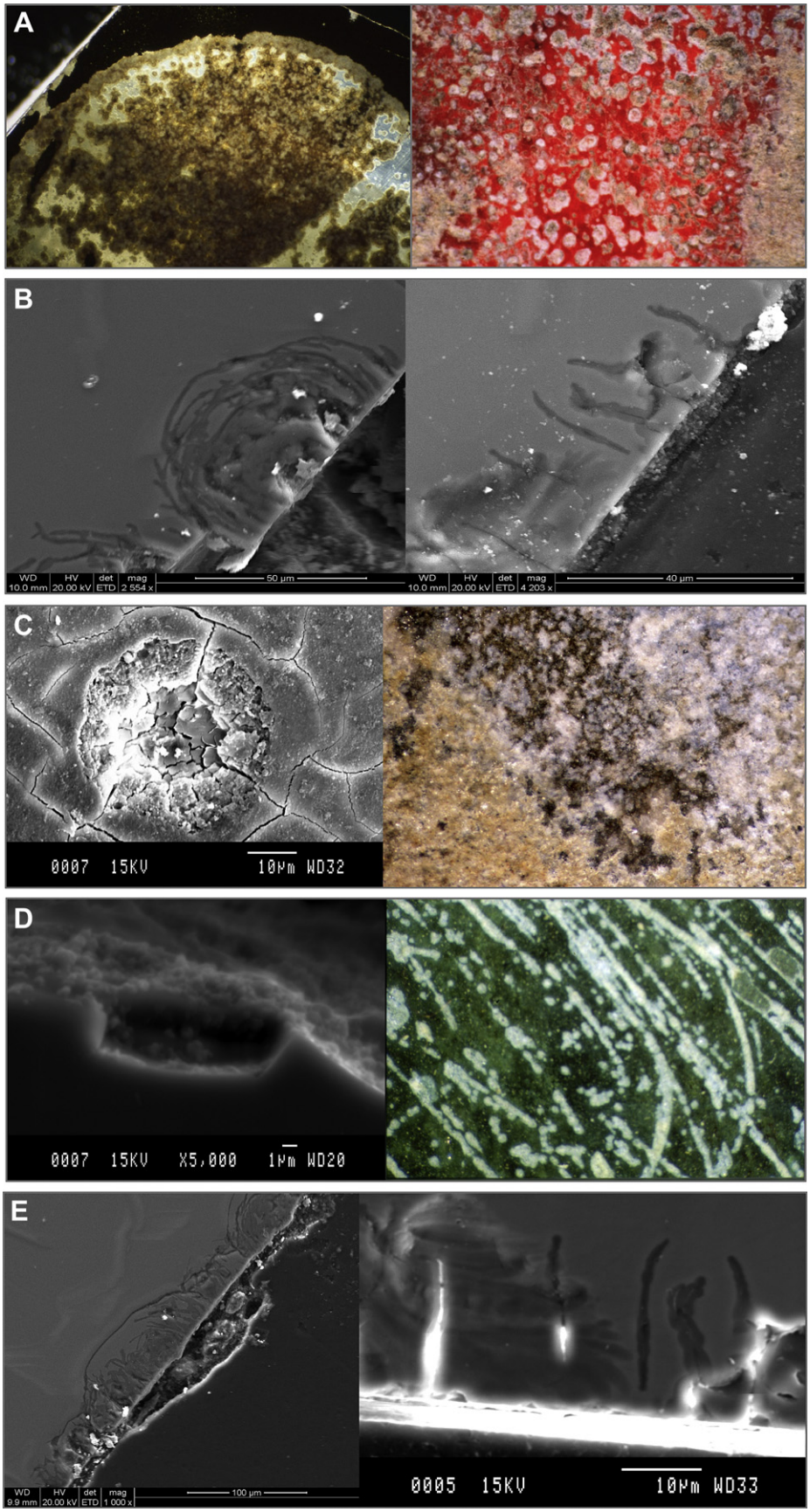

Fig. 3. Patina, leaching surface, and bio-pitting observed on glass samples. 3A: Macroscopic pictures corresponding to two Tarragona glass samples. Left image shows silica-leached surface directly in contact with glass and before patina (orange at bottom and more dark at top) developed in sample T-5. Right image shows detail of patina and bio-pitting

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instrumental error. Zoned glasses (i.e., red glasses consisting of a deep red plate sandwiched between white glass) were also studied by acquisition of a profile of points orthogonal to the planar optical anisotropies. The analytical accuracy and precision was also controlled by means of internal standards (Brill, 1999).

2.3. Molecular analysis

2.3.1. DNA extraction from glass samples

Extraction of DNA was performed directly from the glass samples using the method previously described by Sert and Sterflinger (2010) with the following modifications: Prior to lysis, a piece of glass (0.2–0.25 g) was triturated with a mortar and the obtained powder, together with 500 μl lysing buffer, was added to the tubes of the lysing matrix E (MP Biomedicals). The mixture was shaken in a cell disrupter (Thermo Savant FastPrep, FP120, Holbrook, USA) at full speed for 40 s, and incubated for 1 h at 65 °C. Afterward, the mixture was shaken again at full speed for 40 s and then centrifuged for 10 min at 10,000 g. The supernatant was transferred to a new Eppendorf tube and an approximately equal volume of chloroform/isoamyl alcohol was added, mixed thoroughly, and centrifuged for 5 min in a microcentrifuge. This step was repeated using phenol/chloroform/isoamyl alcohol. The supernatant was transferred to a new Eppendorf tube and further purified using the QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. The final elution step was repeated twice with 100 ml of 80 °C preheated ddH2O (Sigma Aldrich, St. Louis, USA). The purified DNA was used directly for PCR amplification.

2.3.2. PCR amplification of extracted DNA

For the analysis of fungal sequences, fragments of about 700 bp in size corresponding to the ITS1, the ITS2 region, and the adjacent 5.8S rRNA gene, were amplified with the primer pair ITS1 and ITS4 (White et al., 1990). For DGGE analysis, a nested PCR was performed with the PCR product of the first round as template DNA using the primers ITS1GC with a 37-base GC clamp attached to the 5′ end (Muyzer et al., 1993) and ITS2. All reactions were carried out as described in Michaelsen et al. (2006).

For the identification of bacterial 16S rRNA sequences, DNA was amplified with the primer pair 341f/907r (Muyzer et al., 1993; Teske et al., 1996). For DGGE analysis, 200-bp fragments of the 16S rDNA were amplified with a nested PCR using the eubacterial specific primer 341f-GC with a 40-bp GC clamp added to its 5′ end (Muyzer et al., 1993) and the universal consensus primer 518r (Neefs et al., 1990). PCR conditions were as described by Schabereiter-Gurtner et al. (2001a). All PCR products were analyzed by electrophoresis in a 2% (w/v) agarose gel.

2.3.3. Denaturing gradient gel electrophoresis (DGGE)

The DGGE was performed as previously described (Muyzer et al., 1993) using a D-Code system (BioRad) in ×0.5 TAE (20 mM Tris, 10 mM acetate, 0.5 mM Na2 EDTA; pH7.8 with 8% (w/v) acrylamide). Gels were run at a constant temperature of 60 °C with a voltage of 200 V during 3.5 h and 6 h, respectively, for bacterial and fungal fingerprints. The linear chemical gradient of denaturants used in this study [100% denaturing solution contains 7 M urea and 40% (v/v) formamide] are indicated in the figures.

After completion of electrophoresis, gels were stained in a 1 μg ml^{-1} ethidium bromide solution (stock: 10 mg ml^{-1}) for 20 min and afterward visualized by a UVP documentation system (BioRad Transilluminator, Universal Hood; Mitsubishi P93D-printer).

2.3.4. Creation of clone libraries and sequence analysis

To obtain a detailed phylogenetic identification of the microbial community members, clone libraries containing either ITS fungal regions (fungal community) or 16S rRNA fragments (bacterial community) were carried out. For fungal clone libraries, the DNA template was amplified using the primers ITS1/ITS4 as mentioned above. For bacterial clone libraries, the primer pair 341f/907r was used as mentioned above. The PCR products were purified using the QIAquick PCR Purification Kit Protocol (Qiagen, Hilden, Germany) and resuspended in ddH2O water.

Purified PCR products were ligated into the pGEM-T easy Vector system (Promega, Vienna, Austria) following the instructions of the manufacturer. The ligation products were transformed into One-shot TOP10 cells (Invitrogen). These cells allow the identification of recombinants (white colonies) on an indicator LB medium containing ampicillin (100 μg ml^{-1}), streptomycin (25 μg ml^{-1}), and X-Gal (5-bromo-4-chloro-3-indolyl-ß-1-galactopyranoside; 0.1 mM) (Sambrook et al., 1989).

Clones were screened in a DGGE gel and sequenced as described by Schabereiter-Gurtner et al. (2001a). Comparative sequence analysis was performed by comparing pair-wise insert sequences with those available in the public online database NCBI using the BLAST search program (Altschul et al., 1997). The resulting sequences of the bacterial and fungal clones have been deposited at GenBank: Genetic sequence database at the National Center for Biotechnical Information (NCIB) (GenBank ID: BA123456) (Tables 3and 4).

3. Results

3.1. Patinas, leached surface, and glass characterization

Stereomicroscope observations showed different situations on each glass sample. All glass samples, except T-28 (Cathedral of Tarragona) and STM-13 (church of Santa Maria del Mar), were covered by discontinuous orange and/or beige patina (Figs. 1C and 3A and C). In addition, glasses presented traces of biological activity as bio-pitting (Fig. 3A and B), isolate micro-cracks and some interconnected micro-cracks developed in glass, in patina, in old paints, or in grisaille (Fig. 3C).

Some samples showed a leached surface (Figs. 1C and 3A left) and also evidence of a gel with a silica composition, as determined by SEM. The more external part of the patina showed to be orange and the bottom beige (Fig. 3A, left). Occasionally, the patina was formed in glass surfaces previously attacked by microorganisms (Fig. 3A), producing a more or less penetrating pitting into the glass (Fig. 3B). This destroyed surface can also be filled (constructive activity) with authigenic minerals (patina).

developed in red glass plaqué in sample T-6. 3B: SEM analyses on sample T-6 show the degree of penetration of this activity into the glass. 3C: Orange and beige patina developed in red plaqué glass (sample T-35). Glass and patina have been affected by biological activity observed as small pits. The SEM picture shows the pit evolution to inner part. 3D: Decayed surface corresponding to sample STM-16 from Santa Maria del Mar. The right image shows circles isolated or one after the other forming a row arranged in a preferential direction. Occasionally there is an alignment perpendicular to this main direction. The left image, observed with microscopy in the thin section perpendicular to the glass surface, shows the pit penetration in the green glass zone. This penetration was short, due to the toxicity of the saturated copper, chromophore element found in this glass (3.71% CuO). 3e: SEM pictures of K-glass from Tarragona (T-5) showing advanced glass decay caused by bio-pitting; note the curved-branched irregular pitting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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SEM observations of the glass corroborated the morphology and evolution of pits (Fig. 3B and D), and the advanced glass decay. Fig. 3D shows initial development of curved-branched irregular pitting not directly related to cracking surface. Each pit appears as an incipient nodule-like glass decay form overprinted on the curved branch of pit progression. These branches of pits tend to coalesce and provide a deeply penetrated upper film of the glass, sometimes associated with an external surface patina. We could also observe that in plaqué glass the bio-pitting stopped when the microorganisms arrived at the colored layers, which contain heavy metals – e.g., copper – that are noxious to microorganisms (Fig. 3C). The diameter of the pit holes decreases from the external (50 μm) to the internal part (20 μm, Fig. 3C). This activity was also observed in the K-rich glass STM-16 (Fig. 3D). Fig. 3E shows a macroscopic photograph with a bio-pitting arrangement made in the glass surface, and SEM shows the detail of the pit hole section related to organic activity.

The mineralogical composition of patinas, determined by XRD and corroborated by SEM-EDAX analyses, revealed the presence of gypsum ($CaSO_4 \cdot 2H_2O$), bixbyite (Mn_2O_3), syngenite [$K_2Ca(SO_4)_2 \cdot H_2O$], thenardite [(α-Na_2SO_4)], calcite ($CaCO_3$), and quartz (SiO_2), as well as the presence of clay minerals and Ca-oxalate (weddellite, $CaC_2O_4 \cdot 2H_2O$, and whewellite, $CaC_2O_4 \cdot H_2O$), determined in orange patina.

The chemical composition of the investigated samples obtained from the Cathedral of Tarragona and the church of Santa Maria del Mar are showed in Tables 1and 2, respectively. In all samples the vitrifying compound was SiO_2. Depending on the flushing compound type used, K_2O or Na_2O, we distinguish two main compositional groups of glasses: Na-rich and K-rich (or K–Ca-rich). The Na-rich glasses typically show greater silica content (>68%) than the K-rich or K–Ca-rich glasses (around 50%). The other network-forming, Al_2O_3, has a uniform value ~2% independently of the K- or Na-rich character. The K-group glasses always contain another oxide, P_2O_5 (3–4%), which is absent in the Na-group glasses. Three (T-6, T-31, and T-35) out of six samples from the Cathedral of Tarragona corresponded to red plaqué glass, formed by red layer between 160 and 200 μm thick and colorless 2.5 mm. The color exhibited can be due to the presence of CuO (comparing the results in Table 1). Two of the five samples from Santa Maria del Mar were plaqué as well, STM-16 green and STM-19 blue. The first one was double plaqué in cross section, consisting of a 60-μm-thick layer that was colorless, one layer green to 720 μm, another colorless to 720 μm, other green and colorless also to ca. 720 μm. The main chemical compounds are the same, but the green color is due to enriched Cu, ~3.7% CuO; when this value is >1% the color obtained is green. The blue plaqué was formed by blue glass of a 150-μm-thick layer and other colorless to 2.8 mm. Comparing the oxide constituents among the blue and colorless layers, differences were observed in Mn and Fe, showing higher values in the blue zones.

3.2. Molecular analysis

For molecular analyses, pieces of glass (0.2–0.25 g) were used for direct DNA extraction. The DNA extracts were amplified by PCR with primers targeting the 16S rRNA gene of bacteria, as well as the ITS regions of fungi. The bacterial and fungal amplified fragments were further analyzed by DGGE analysis, revealing fingerprints for both bacterial and fungal communities.

Fig. 4 shows the DGGE profiles derived from the bacterial and fungal communities colonizing the samples obtained from the Cathedral of Tarragona and the church of Santa Maria del Mar in Barcelona. In general, bacterial DGGE profiles showed a higher complexity in the community structure, with many dominant bands as well as some other faint bands (Fig. 4A). The DGGE profiles derived from the fungal communities showed fewer bands, indicating a lower biodiversity of fungi on the glass samples (Fig. 4B).

To obtain a phylogenetic identification of the individual members of the bacterial and fungal communities inhabiting the glasses of the two investigated locations, two K-rich glass samples (one from each location) were selected for the creation of clone libraries containing either the bacterial 16S rRNA gene or the fungal ITS regions and the 5.8S rRNA gene. The resulting bacterial and fungal clones were further screened by DGGE and clones were selected for sequencing. The obtained sequences were compared with 16S rRNA gene sequences and ITS regions of known bacteria and fungi, respectively, listed in the EMBL database. Figs. 5and 6 show the DGGE profiles derived from bacterial (A) and fungal (B) clones selected for sequencing obtained from samples T-5 (Cathedral of Tarragona) and STM-16 (church of Santa Maria del Mar), respectively. Tables 3and 4 show the phylogenetic affiliations of the bacterial and fungal clones, respectively, obtained from both samples.

3.3. Microflora associated with the glass material

3.3.1. Bacteria

Table 3 shows the phylogenetic affiliations of all bacterial clones obtained in this study. A total of 19 and eight bacterial sequences were obtained from samples T-5 and STM-16, respectively.

From sample T-5 (Table 3A), nine sequences (47.3% of the bacterial sequences obtained from this sample) showed high score similarities, ranging from 90 to 100%, with members of the Proteobacteria (Alpha-, Beta-, and Gamma-proteobacteria) being related to species of the genera *Methylobacterium* (K59), *Bradyrhizobium* (K91), *Ralstonia* (K15), *Delftia* (K67), *Variovorax* (K93), *Acinetobacter* (K79), and *Thermomonas* (K90), as well as to two

Table 1
Chemical composition of the glass samples obtained from the Cathedral of Tarragona.

Sample	T-3	T-5	T-6		T-28	T-31		T-35	
Color	Blue	Brown–yellow	Red	Colorless	Purple	Red	Colorless	Red	Colorless
SiO_2	48.78	49.09	48.69	47.71	67.61	49.27	48.97	46.68	43.42
K_2O	16.08	18.75	18.10	19.04	4.54	19.09	19.10	19.59	21.41
Na_2O	0.35	0.30	0.43	0.39	15.83	0.36	0.35	0.46	0.58
P_2O_5	3.83	3.97	3.74	4.40	0.66	4.39	4.25	3.88	4.30
CaO	19.02	17.66	17.84	19.03	7.42	17.78	17.37	19.28	20.78
MgO	4.98	4.82	4.99	5.10	0.25	4.75	4.62	5.05	4.74
Al_2O_3	2.33	2.20	2.36	2.42	2.04	2.30	2.27	2.59	2.48
MnO	1.24	1.18	0.98	1.12	0.96	1.01	1.00	1.20	1.34
FeO	1.07	0.59	0.44	0.47	0.50	0.53	0.54	0.53	0.58
CuO	0.26	0.18	0.95	0.12	0.03	0.32	0.04	0.44	0.07
PbO	0.04	0.00	0.06	0.04	0.02	0.01	0.01	0.09	0.11
TiO_2	0.17	0.15	0.05	0.15	0.11	0.17	0.16	0.18	0.18

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Table 2
Chemical composition of the glass samples obtained from the church of Santa Maria del Mar. Barcelona.

Sample	STM-13	STM-16				STM-19		STM-30
Color	Yellow	Green	Colorless	Green	Colorless	Blue	Colorless	Purple
SiO_2	65.07	49.44	50.81	49.23	50.55	65.28	68.91	63.76
K_2O	2.52	17.82	18.14	17.44	18.38	2.22	0.36	1.91
Na_2O	17.331	1.34	1.22	1.27	1.24	12.89	17.07	15.72
P_2O_5	0.59	4.82	5.39	4.96	5.12	0.02	0.04	0.04
CaO	7.14	13.90	14.55	13.74	14.41	12.30	10.79	11.87
PbO	0.07	0.08	0.10	0.00	0.06	0.00	0.13	0.01
MgO	2.86	4.69	4.90	4.59	5.00	0.30	0.59	0.05
Al_2O_3	1.93	2.00	2.05	1.91	2.17	1.29	0.92	2.41
MnO	0.7	1.16	1.24	1.06	1.26	3.93	0.01	2.81
FeO	0.74	0.54	0.51	0.58	0.57	0.76	0.12	0.17
CuO	0	3.71	0.09	3.82	0.03	0.01	0.03	0.03

uncultured bacterial clones (K95 and K83). Three sequences of sample T-5 (15.8%) affiliated with members of the Bacteroidetes phylum, namely with *Prevotella* sp. (K61) and Bacteroidetes bacteria (K76 and K78). Five sequences (26.3%) affiliated with members of the Actinobacteria phylum, with species of the genera *Kocuria* (K24 and K58), *Micrococcus* (K56), and *Cellulomonas* (K63), as well as with an uncultured Actinobacterium clone (K66). One single sequence (5.3% of bacterial sequences) showed itself to be related to the Firmicutes phylum, namely with a marine bacterium (K68), and finally clone K33 (5.3%) was affiliated with a non-classified uncultured bacterium clone.

Table 3B shows the phylogenetic affiliations of bacterial clones derived from sample STM-16. Two sequences (25% of the bacterial sequences obtained from this sample) showed high score similarities, ranging from 96 to 99%, with members of the Beta-Proteobacteria, namely with species of the genera *Delftia* (K96 and K97). One single sequence of sample STM-16 (12.5%) affiliated with members of the Bacteroidetes phylum, namely with an uncultured Bacteroidetes bacterium clone (K1). Five sequences (62.5%) affiliated with members of the Actinobacteria phylum, with species of the genus *Arthobacter* (K12, K13, K14, K66, and K71).

3.3.2. Fungi

Table 4 shows the phylogenetic affiliations of all fungal clones obtained in this study. A total of seven and four fungal sequences were obtained from samples T-5 and STM-16, respectively.

From sample T-5 (Table 4A), four sequences (57.1% of the fungal sequences obtained from this sample) showed a high level of similarity (99–100%) with *Cladosporium* spp. (F20, F61, F107, and F109). One sequence (14.3%) showed the maximum score similarity (100%) with *Eurotium amstelodami* (F11), and the remaining two sequences (28.6%) showed similarities with *Penidiella venezuelensis* strains (clones F7 and F113).

Table 4B shows the phylogenetic affiliations of fungal clones derived from sample STM-16. Three out of the four obtained sequences (75% of the fungal sequences obtained from this sample)

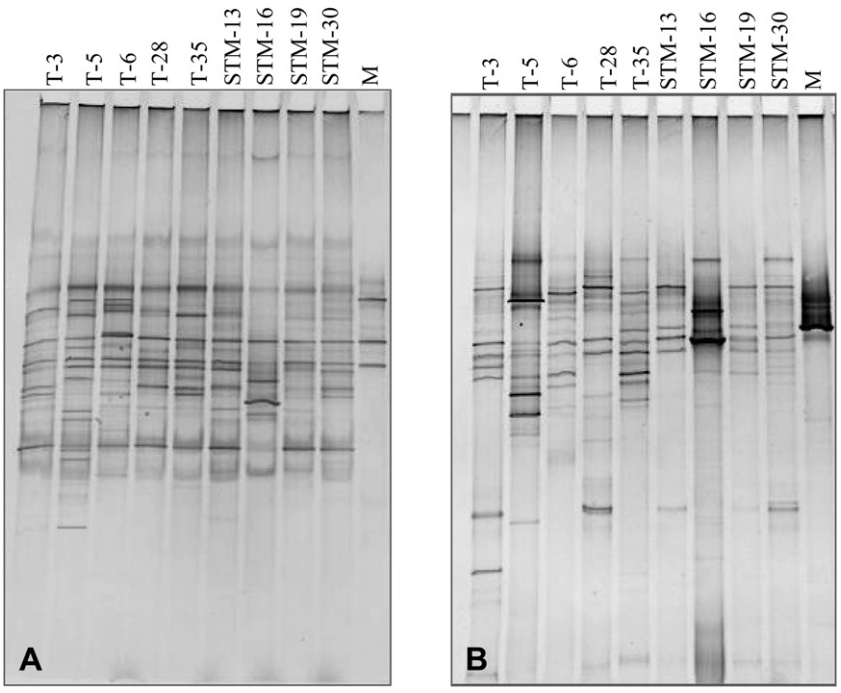

Fig. 4. DGGE fingerprints of DNA fragments encoding bacterial 16S rDNA, using a linear gradient of denaturants of 25–60% (A) and the fungal ITS1 region, using a linear gradient of denaturants of 20–50% (B) derived from original stained glass window samples of the Cathedral of Tarragona and the church of Santa Maria del Mar. Numbers of lanes represent the different glass samples. T: Tarragona; STM: Santa Maria del Mar. M. marker: a mixture of *Bacillus pumilus*, *Pseudomonas stutzeri*, and *Myxococcus xanthus* (A), *Cladosporium* sp. (B).

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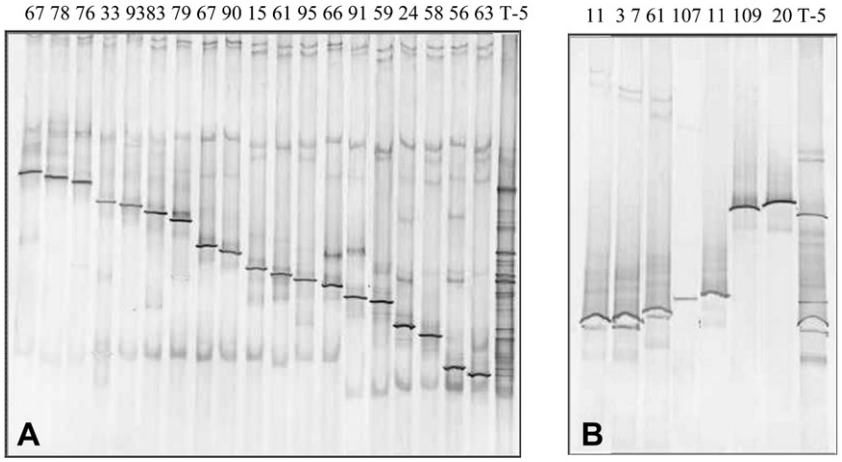

Fig. 5. DGGE fingerprints of DNA fragments encoding the bacterial 16S rDNA, using a linear gradient of denaturants of 25–55% (A) and the fungal ITS1 region, using a linear gradient of denaturants of 20–40% (B) derived from sample T-5 of the Cathedral of Tarragona, as well as the profiles of sequenced clones. Nomenclature indicates the numbers of clones, as indicated in Tables 3and 4.

showed high score similarities (98–99%) with *Phoma* sp. (Clones F1, F18, and F45); the remaining sequence affiliated with species of the genus *Cladosporium* (clone F5).

4. Discussion

4.1. Biodeterioration of glass

As reported in previous works (Garcia-Valles and Vendrell, 2002; Garcia-Valles et al., 2003), the glasses made with potash flux (K-rich composition) are more easily decayed than those with a Na-rich composition. The microscopic analyses revealed that the only visible deterioration phenomenon was related to microbial activity. Indeed, there are several studies (Callot et al., 1987; Krumbein et al., 1991, 1995; Drewello and Weissmann, 1997) highlighting the relevance of microbial activity in the alteration of glasses.

The biological attack observed in this study resulted in decreasing of transparency of the glasses, in combination with the appearance of orange patinas. In addition, the presence of biopitting – more or less developed depending on the glass composition – was observed. Previous work (Koestler et al., 1986) already reported that pitting occurred only on glass with a K-rich composition but not on Na-glass.

In some external areas of the analyzed glasses, it was possible to recognize a layer of new mineral phases. The thickness of this layer depends on both the glass composition and the environmental conditions (Garcia-Valles et al., 2003; Aulinas et al., 2009). In these areas we found remains of microbial activity. The biogenic formation of calcite, weddellite, and gypsum has been shown to be influenced by the microorganisms inhabiting the surfaces (Krumbein, 1986; Garcia-Valles et al., 1996, 1997). In Mediterranean areas, significant changes in the ecological dynamics of microorganisms colonizing rock surfaces occur due to seasonal and

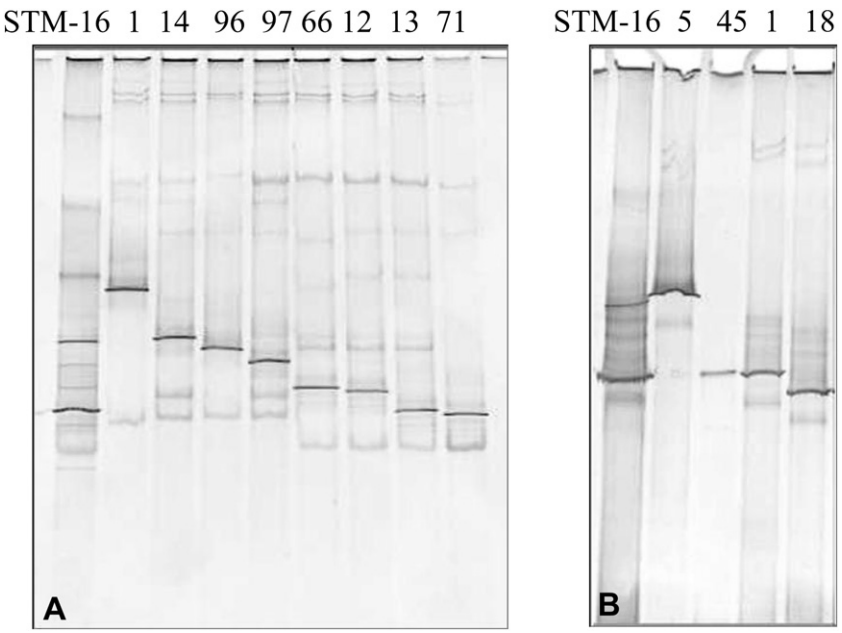

Fig. 6. DGGE fingerprints of DNA fragments encoding the bacterial 16S rDNA, using a linear gradient of denaturants of 25–55% (A) and the fungal ITS1 region, using a linear gradient of denaturants of 20–40% (B) derived from sample STM-16 of the church of Santa Maria del Mar, as well as the profiles of sequenced clones. Nomenclature indicates the numbers of clones as indicated in Tables 3and 4.

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Table 3
Phylogenetic affinities of partial 16S rRNA coding sequences detected in stained glass windows. 3A) Sequences derived from sample T-5 from the cathedral of Tarragona, 3B) Sequences derived from sample STM-16 from the church of Santa Maria del Mar, Barcelona.

Phylum	Clone Nr.	Length (bp)	Closest identified phylogenetic relatives [EMBL accession numbers]	Simil. (%)	Accession Nr.
(A)					
Gamma-Proteobacteria	K33	[250]	Acinetobacter sp. N22 [JQ687406].	97	JF812117
Alpha-Proteobacteria	K59	[320]	*Methylobacterium* sp. F37 gene for 16S rRNA, strain: F37 [D32234] chlorine-resistant (Hiraishi et al. 1995).	99	JF812120
			Methylobacterium sp. 16S rRNA gene [Z23159] resistant to dessication.	99	
	K91	[562]	*Bradyrhizobium elkanii* strains 16S ribosomal RNA gene, partial sequence [HQ533233, HQ533234, HQ533235, HQ533239, HQ533240, HQ533241, HQ533242, HQ533247].	98	JF812131
			Bradyrhizobium sp. 16S ribosomal RNA gene, partial Sequence [HQ704812, HQ704813, HQ704814, HQ704815].	98	
	K95	[588]	Uncultured bacterium clone BSS37 16S ribosomal RNA gene, partial Sequence [HQ397481] from coastal saline soils.	91	JF812133
Beta-Proteobacteria	K15	[587]	*Ralstonia pickettii* strain QL-A6 16S ribosomal RNA gene, partial Sequence [HQ267096].	99	JF812115
			Ralstonia sp. R1-24 16S ribosomal RNA gene, partial sequence [HQ436435] isolated from a lake.	99	
	K67	[602]	Uncultured *Delftia* sp. partial 16S rRNA gene, clone [FN555650] inhabiting deteriorated stones of the Closter Royal Hospital in Granada, Spain (Ettenauer et al. 2011).	98	JF812124
			Delftia acidovorans strain CL-9.07 16S ribosomal RNA gene, partial sequence [HQ113205].	98	
	K93	[587]	*Variovorax paradoxus* strain BS244 16S ribosomal RNA gene, partial Sequence [HQ005420, HQ005423].	95	JF812132
Gamma-Proteobacteria	K79	[589]	*Acinetobacter* sp. enrichment culture clone B1-5 16S ribosomal RNA gene, partial sequence [GU570643] a cold-adapted bacteria.	100	JF812128
			Acinetobacter sp. 2009I8 16S ribosomal RNA gene, partial sequence [GU290325] bio-degumming microbiology.	100	
			Uncultured *Acinetobacter* sp. clone 3P-4-1-D04 16S ribosomal RNA gene, partial sequence [EU706031] Microbial Burden and Community Analyses of Phoenix Spacecraft Assembly.	100	
	K83	[590]	Uncultured soil bacterium clone TA8 16S ribosomal RNA gene, partial Sequence [DQ297940] from hydrocarbon contaminated soil.	90	JF812129
	K90	[589]	*Thermomonas dokdonensis* strain DS-58 16S ribosomal RNA gene, partial sequence [EF100698] isolated from soil.	93	JF812130
Bacteroidetes	K61	[581]	*Prevotella* sp. clones 16S ribosomal RNA gene, partial Sequence [AY005065, AM420217].	99	JF812121
	K76	[383]	Bacteroidetes bacteria 16S ribosomal RNA gene, partial sequence [EU559746, FJ816610] from Lake Michigan water	95	JF812126
			Sediminibacterium sp. strains, partial 16S rRNA gene, [AM990455, AM990455] from industrial cooling water system.	95	
	K78	[580]	Bacteroidetes bacteria 16S ribosomal RNA gene, partial sequence [EU559746, FJ816610] from Lake Michigan water.	99	JF812127
			Sediminibacterium sp. strains, partial 16S rRNA gene, [AM990455] from industrial cooling water system.	99	

(*continued on next page*)

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Table 3 (*continued*)

Phylum	Clone Nr.	Length (bp)	Closest identified phylogenetic relatives [EMBL accession numbers]	Simil. (%)	Accession Nr.
Actinobacteria	K24	[570]	*Kocuria aegyptia* strain YIM 70003 16S ribosomal RNA gene, partial sequence [DQ059617] isolated from a saline, alkaline desert soil in Egypt (Li et al. 2006).	96	JF812116
			Kocuria sp. HI-A4a 16S ribosomal RNA gene, partial sequence [DQ205297], culturable microbial diversity and the impact of tourism in Kartchner Caverns, Arizona (Ikner et al. 2007).	96	
	K58	[570]	Uncultured *Kocuria* sp. partial 16S rRNA gene, clone F-K34 [FN555669] inhabiting deteriorated stones of the Royal Hospital in Granada, Spain (Ettenauer et al. 2011).	99	JF812119
			Kocuria rosea strains 16S ribosomal RNA gene, partial Sequences [HQ830206, HQ538755, HQ202874].	99	
	K56	[409]	*Micrococcus* sp. RNE 20 partial 16S rRNA gene, strain RNE 20 [FR749831] Microbiological induced corrosion in nuclear materials (Forte-Giacobone et al. 2011).	97	JF812118
	K63	[570]	*Cellulomonas* sp. Mn5-4 16S ribosomal RNA gene, partial sequence [HQ730135] isolated from copper iron ore in China.	99	JF812122
	K66	[601]	Uncultured *Actinobacterium* clone JBS_5Y17 16S ribosomal RNA gene, partial sequence [EU702777] from benthic bacterial community.	89	JF812123
Fimicutes	K68	[487]	Marine bacteria gene for 16S ribosomal RNA, partial sequence [AB607166; AB607142; AB607143].	98	JF812125
			Enterococcus sp. 16S ribosomal RNA gene, partial [HQ677826, AB596997, HM584103].	98	
(B)					
Beta-Proteobacteria	K96	[587]	*Delftia acidovorans* SPH-1, complete genome [CP000884].	99	JF812140
	K97	[587]	Uncultured *Delftia* sp. partial 16S rRNA gene, clones [FN435923, FN435934, FN435975, FN434405, FN434411, FN434425] inhabiting deteriorated stones of the Closter of San Jeronimo and the Royal Hospital in Granada, Spain (Ettenauer et al. 2011).	96	JF812141
			Delftia sp. R-41380 partial 16S rRNA gene, strain R-41380. [FR682925] with pesticide degradation capability.	96	
			Delftia sp. EK3 16S rRNA gene, isolate EK3. [AJ237966] degradation of 1,3-dichloropropene in industrial waste water.	96	
Bacteroidetes	K1	[580]	Uncultured Bacteroidetes bacterium clone A3-01QJH 16S ribosomal RNA gene, partial sequence [EU434886] from microbial communities colonizing a single natural shelter containing prehistoric paintings (Portillo et al. 2009).	100	JF812134
Actinobacteria	K12	[570]	*Arthrobacter* sp. 16S ribosomal RNA gene, partial sequences [GU574112, GU574114, GU574116] from mould-colonized water damaged building material (Schäfer et al. 2010).	99	JF812135
			Arthrobacter sp. M71_S08 partial 16S rRNA gene, isolate M71_S08 [FM992748] from the deep oligotrophic Eastern Mediterranean Sea (Gärtner et al. 2011).	99	
	K13	[570]	*Arthrobacter* sp. 16S ribosomal RNA gene, partial sequences [GU574112, GU574114, GU574116] from mould-colonized water damaged building material (Schäfer et al. 2010).	99	JF812136
			Arthrobacter sp. M71_S08 partial 16S rRNA gene, isolate M71_S08 [FM992748] from the deep oligotrophic Eastern Mediterranean Sea (Gärtner et al. 2011).	99	
	K14	[590]	Uncultured soil bacterium clone TA8 16S ribosomal RNA gene, partial Sequence [DQ297940] from hydrocarbon contaminated soil.	93	JF812137
			Arthrobacter sp. WPCB182 16S ribosomal RNA gene, partial sequence [FJ006921].	92	
	K66	[570]	*Arthrobacter* sp. 16S ribosomal RNA gene, partial sequences [GU574112, GU574114, GU574116] from mould-colonized water damaged building material (Schäfer et al. 2010).	99	JF812138
			Arthrobacter sp. M71_S08 partial 16S rRNA gene, isolate M71_S08 [FM992748] from the deep oligotrophic Eastern Mediterranean Sea (Gärtner et al. 2011).	99	
	K71	[570]	*Arthrobacter* sp. 16S ribosomal RNA gene, partial sequences GU574114, GU574116] from mould-colonized water damaged building material (Schäfer et al. 2010).	99	JF812139
			Arthrobacter sp. M71_S08 partial 16S rRNA gene, isolate M71_S08 [FM992748] from the deep oligotrophic Eastern Mediterranean Sea (Gärtner et al. 2011).	99	

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Table 4
Phylogenetic affinities of the fungal ITS coding sequences detected in stained glass windows. 4A) Sequences derived from sample T-5 from the cathedral of Tarragona, 4B) Sequences derived from sample STM-16 from the church of Santa Maria del Mar, Barcelona.

Phylum	Clone	Length (bp)	Closest identified phylogenetic relatives [EMBL accession numbers]	Simil. (%)	Accession Nr.
(A)					
Ascomycota	F7	[554]	*Penidiella venezuelensis* strain CBS 106.75 [EU019278].	94	JF812146
			Uncultured fungal clones [GU073011, GU073016] from fungal communities in sandstone.	93	
	F11	[410]	*Eurotium amstelodami* culture-collection NRRL:62022 [HM751091].	100	JF812147
	F20	[553]	*Cladosporium cladosporioides* [EF577236].	100	JF812148
			Cladosporium sphaerospermum [HQ248189].	100	
			Uncultured fungus clone L042884-122-064-C08 [GQ851693] from continental and marine air.	100	
	F61	[552]	*Cladosporium cladosporioides* [EF577236].	90	JF812149
			Cladosporium sphaerospermum [HQ248189].	90	
			Uncultured fungus clone L042884-122-064-C08 [GQ851693] from continental and marine air.	90	
	F107	[454]	*Cladosporium cladosporioides* [EF577236].	99	JF812150
			Cladosporium sphaerospermum [HQ248189].	99	
			Uncultured fungus clone L042884-122-064-C08 [GQ851693] from continental and marine air.	99	
	F109	[553]	*Cladosporium cladosporioides* [EF577236].	99	JF812151
			Cladosporium sphaerospermum [HQ248189].	99	
			Uncultured fungus clone L042884-122-064-C08 [GQ851693] from continental and marine air.	99	
	F113	[553]	*Penidiella venezuelensis* strain CBS 106.75 [EU019278].	94	JF812152
			Uncultured fungal clones [GU073011, GU073016] from fungal communities in sandstone.	93	
(B)					
Ascomycota	F1	[540]	Uncultured fungus clone LX037622-122-003-G10 [GQ999376] from continental and marine air.	99	JF812142
			Phoma glomerata isolate XSD-41 [EU273521].	99	
			Phoma sp. [HQ630999, GU566295, FJ950743] from plants and soils.	99	
	F5	[553]	*Cladosporium cladosporioides* [EF577236].	99	JF812143
			Cladosporium sphaerospermum [HQ248189].	99	
			Uncultured fungus clone L042884-122-064-C08 [GQ851693] from continental and marine air.	99	
	F18	[540]	Uncultured fungus clone LX037622-122-003-G10 [GQ999376] from continental and marine air.	99	JF812144
			Phoma glomerata isolate XSD-41 [EU273521].	99	
			Phoma sp. [HQ630999, GU566295, FJ950743] from plants and soils.	99	
	F45	[300]	Uncultured fungus clone Alb_O_AugF04 GU174372 from hardwood forests.	98	JF812145
			Phoma sp. [HM751088, AB369463, AB369459].	98	

environmental variations, and they contribute to mineral deposition (Garcia-Valles et al., 2010). The similar environmental conditions of the two investigated locations, including proximity to the sea (humidity, mist), pollution, and the chemical composition of the windows, result in the same leaching corrosion products on these glasses: the formation of a layer of silica gel and the leaching of potassium.

Potassium and calcium produce hydrated sulfates of K and Ca [e.g., syngenite, $K_2Ca(SO_4)_2H_2O$ and gypsum $CaSO_4H_2O$, and sodium generates thenardite (Na_2SO_4)].

4.2. Micro-biota associated with the biodeterioration phenomena

The results derived from molecular analyses showed the presence of complex bacterial communities colonising the decayed glasses of the Cathedral of Tarragona and the church of Santa Maria del Mar. The identified bacterial clones grouped into three main phyla – Proteobacteria, Bacteroidetes, and Actinobacteria – in both locations, as well as members of the Firmicutes in the case of the Cathedral of Tarragona. Comparing the bacterial communities detected on the glasses of both investigated locations, the diversity of sample T-5 from the Cathedral of Tarragona was higher than that observed in sample STM-16 from the church of Santa Maria del Mar (see Figs. 5A and 6A). Furthermore, members of the Proteobacteria dominated at the first location (47.3% of the bacterial sequences of this sample), while members of the Actinobacteria were seen to be dominant at the church of Santa Maria del Mar (62.5% of the bacterial sequences of this sample).

Interestingly, many of the detected bacteria were phylogenetically related to species well known for their ability to precipitate minerals, such as *Delftia* sp., *Methylobacterium* sp., *Variovorax* sp., *Arthrobacter* sp., *Kocuria* sp., and *Micrococcus* sp., as well as some Bacteroidetes bacteria (Jroundi et al., 2010; Ettenauer et al., 2011), which could explain the presence of mineral precipitation on the glass surfaces. Furthermore, some species of the detected genera are orange–pink–red pigmented bacteria, such as *Methylobacterium* sp., *Arthobacter* sp., *Kocuria* sp., and *Micrococcus* sp. (Ventosa et al., 1993; Hiraishi et al., 1995; Heyrman et al., 2005). These bacteria could be responsible of the appearance of the orange patinas on the glasses, together with the presence of the Ca-oxalates (weddellite and whewellite) found in the patinas, which can be yellow, or yellowish-brown to brown.

The fungal communities detected in both locations were shown to be less diverse than those of bacteria, but again, the biodiversity was higher in the sample obtained from the Cathedral of Tarragona, with a dominance of *Cladosporium* sp. By contrast, species of the genus *Phoma* dominated on sample STM-16 from the church of Santa Maria del Mar.

The bio-pitting observed with SEM analyses could be directly related to the microorganisms able to produce mycelia, such as the Actinobacteria and the filamentous fungi detected in this study. However, it should be emphasized that the detected fungi are ubiquitous airborne fungi that may be considered more as contaminant than as real glass-inhabiting fungi. The observed pitting could be interpreted as well as the result of fungal attack by other more specialized fungal species in the past and not detectable at the present. However, we cannot determine a clear chronology of biodeterioration. Indirect evidence provided during recent restoration strongly suggests that the biodeterioration of the investigated Catalonian glasses started before industrial and car-induced contamination occurred in these urban settlements (That is, prior biodegradation is now present in the opposite side of a fragment of glass accidentally inverted during historic restorations.).

We could also observe that the microbial penetration in these glasses was related to their chemical composition. Some elements, such as copper, play a role as inhibitors of the bio-activity. In the red

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flashed glasses (Fig. 3E), the microbial development was stopped when the organisms reached the Cu-rich colored layer and, thus, they stopped their destructive activity. In those samples showing ancient bio-activity, we observed a non-uniform spatial distribution of pitting on the surface of the glasses. In some cases, the bio-pitting formed a pattern of channels like those shown in Fig. 3E.

Last but not least, our sequence results show high similarities with bacteria already found on deteriorated stone monuments (Jroundi et al., 2010; Ettenauer et al., 2011), building materials (Schäfer et al., 2010), caves (Ikner et al., 2007), and prehistoric paintings (Portillo et al., 2009), and associated with the corrosion of materials (Forte-Giacobone et al., 2011). Many of the related microorganisms were previously isolated from stones and artworks in the Mediterranean area (Portillo et al., 2009; Jroundi et al., 2010; Ettenauer et al., 2011) which supports the idea that medieval stained glass biodeterioration in the Mediterranean area shows patterns comparable to those developed on the stone (Garcia-Valles et al., 2003, 2010). This is logical if we think that, in historic buildings, both the rock and the glass have been exposed to the same atmospheric conditions, which makes for similar mechanisms of decay on both materials. Furthermore, taking into consideration that Tarragona and Barcelona are cities located at the Mediterranean seaside, it is not surprising to find sequences related to *Methylobacterium* sp. able to tolerate chloride (Hiraishi et al., 1995), *Kocuria aegyptia* isolated from saline environments (Li et al., 2006), oligotrophic species of the genus *Arthrobacter* isolated from the Mediterranean sea (Gärtner et al., 2011), as well as fungal species detected in marine air (see Table 4).

Comparing our data with previous studies focusing on the biological deterioration of historical glass, we find a lack of studies that combine different methodologies, with very few exceptions (Carmona et al., 2006). To date, published studies have focused on the microscopic and chemical (Garcia-Valles and Vendrell, 2002, 2003) or on the microbial characterization of historical glass samples by culture-dependent techniques (Marvasi et al., 2009). Only a few published works have reported on the molecular characterization of historical glass samples (Rölleke et al., 1999; Schabereiter-Gurtner et al., 2001b; Carmona et al., 2006). In the first two molecular studies mentioned above, the authors investigated glass samples derived from the historical window of the chapel in Stockkämpen, Germany. Rölleke et al. (1999) analyzed the bacterial community of this window via DGGE and 16S rDNA sequence analysis. They identified *Flexibacter* sp., as well as members of the ammonia-oxidizing genus *Nitrosospira* and several members of the Actinobacteria, such as *Arthrobacter*, *Frankia*, and *Geodermatophilus*. Schabereiter-Gurtner et al. (2001b) analyzed the fungal communities inhabiting the same window by 18S rDNA-based DGGE fingerprinting, creation of clone libraries, and sequencing, and compared the detected community with the one obtained from other glass windows located at the church of St. Michael and Johann Baptist in Brakel, Germany. Even though authors found fungal communities consisting of five to eight different fungi on these two glass windows, the molecular strategy they followed was fastidious and very time-consuming, as it was necessary to use at least three different fungal primer combinations to screen 18S rDNA clone libraries. Finally, Carmona et al. (2006) investigated the microbial communities (bacteria and fungi) associated with the historical stained glasses of the Cartuja de Miraflores Monastery (Spain). The bacterial communities were analyzed by 16S rDNA-based DGGE fingerprinting, although no attempt at identification was made. Fungi were identified by cloning and sequencing of the 18S rDNA.

Nowadays it is well known that there is a lack of taxonomic resolution between fungal 18S rRNA genes to closely related taxa and, therefore, this region is not so accurate for an accurate phylogenetic identification of fungi (Anderson and Cairney, 2004). In recent years, ITS regions have become the selected genetic marker for studies on fungal communities in environmental samples (White et al., 1990). For this reason, in previous studies we developed a protocol to obtain DGGE fingerprints derived from fungal ITS regions of DNA directly extracted from different materials of cultural heritage (Michaelsen et al., 2006). To date, we have successfully applied this molecular strategy to analyze fungal communities colonizing paper (Michaelsen et al., 2006, 2009, 2010), stone monuments (Piñar et al., 2009), parchment (Piñar et al., 2011), oil paintings on canvas (Lopez-Miras, personal communication), human remains (Piñar, unpublished data) and, in the present study, glass samples. This strategy has proven easier and more reliable than the one using DGGE fingerprints derived from 18S rDNA fragments.

In addition, with the exception of some few works (Rölleke et al., 1999; Carmona et al., 2006; Marvasi et al., 2009) bacteria were thought not to be dominant colonizers of glass surfaces and not to play a relevant role in the observed deterioration phenomena. The results obtained in the present study clearly show that bacterial communities were more diverse than those of fungi and that the detected bacteria are phylogenetically related to well-known bacteria possessing metabolic activities able to produce the observed biodeterioration of the investigated glasses. This fact highlights the relevance of bacteria, together with fungi, in the biodeterioration of historical glass.

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