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New and old microbial communities colonizing a seventeenth-century wooden church

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Abstract The North of Romania is known for its wooden churches dating from the seventeenth and eighteenth centuries. Their deterioration constitutes a major problem due to their value for the cultural heritage. The microbial community from a seventeenth-century wooden church (Nicula, Romania) was investigated by characterization of uncultivated and cultivated bacteria using 16S rDNA sequence analysis. The study revealed not only the prevalence of the *Bacillus thuringiensis* strain IAM 12077 but also the presence of new microbial communities of *Planomicrobium* and *Variovorax* that were not previously reported in paintings or on wood. The identification of fungi showed the presence of seven common genera found on the walls and icon surfaces. Common bacteria from the human oral microbiota were not identified in the bacterial community.

Introduction

During the last two decades, churches have been an important topic for microbial community studies because of their architectural values and with special focusing on their paintings (Rölleke et al. 1996; Piñar et al. 2009; Capodicasa et al. 2010). Walls, paintings, and even water in the churches were exploited for the identification of the microbial communities inhabiting these niches (Jurado et al. 2002; Radaelli et al. 2004; Portillo et al. 2008; Sterflinger and Pinzari 2012). Most of the microbial deterioration studies have focused on frescoes, mural paintings, and stone monuments (Ciferri 1999; Gaylarde et al. 2011; Imperi et al. 2007; Piñar et al. 2001; Portillo et al. 2009). However, on account of their value, just a few studies describing the biodeterioration agents of paintings on wood panel or canvas have been done, and most of them were control paintings artificially populated with different types of microorganisms (Capodicasa et al. 2010).

A specific characteristic in orthodox churches and rites is "kissing of the icons," a gesture with religious significance that has been mainly focusing on century-old paintings that are being though as having miraculous powers. Kissing and touching icons (paintings on wood or glass) are an important source of additional bacteria. This extra source of microorganisms can contribute to degradation and deterioration of paintings. Furthermore, this practice causes hygienic problems and can contribute to the spread of some pathogenic bacteria from one person to another, taking into account that many sick people visit such places.

The wooden church from Nicula monastery complex (Fig. 1) dates back to the seventeenth century and is declared a national historical monument under the CJ-II-m-A-07722 code. The church was made from oak and was moved to Nicula in 1973 from its initial place at Gostila. It was exposed for more than three centuries to wind, temperature variations, and more importantly, to humidity, especially its external parts. The interior icons are made out of wood and date from the nineteenth century. They do not present a high rate of degradation and, apparently, are in good preservation state.

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Fig. 1 The wooden church from Nicula (Romania)

The aim of this study was the identification of microbial communities inhabiting the Nicula wood church (Cluj County, Romania) and its icons. The investigation also included the associated fungi to bacterial community.

Material and methods

Art work description and sampling

In orthodox churches, the narthex (vestibule) is in direct contact with the external environment. Therefore, to avoid external contamination, our samples were taken from the nave (central part of the church). Nave of the church is separated from the narthex by a wall, and from sanctuary, it is separated by an iconostasis. Lateral walls of the nave have a small window.

Uncultivable microorganism

One sample of degraded wood from a lateral wall (not closed to the window) was taken from the church with the purpose of characterizing the uncultured bacteria.

Cultivable microorganism

On the walls of the church nave, icons are painted on glass and on wood. In the current study, five icons on wood painting were analyzed. Our study included five wooden icons from the nave: two placed on the entrance wall of the nave and three on the iconostasis. Icons were not very close to the door or windows. In order to investigate the bacteria from icons, due to interdiction of sampling wood from these artifacts, the icon surfaces were brushed with rinsed swabs. Two swabs from each icon were stored at 4 °C and plated after 12 h on luria broth (LB) solid media. The plates were incubated at 25 °C.

Cultivable fungi

Six sterile swabs were brushed on the church walls, and one different swab was brushed on each icon. The swabs were used to inoculate Sabouraud media (40 g/L dextrose, 10 g/L peptone, 20 g/L agar, pH 5.6) and incubated for 7 days at 25 °C.

Molecular characterization of the microbial community

DNA extraction and PCR amplification of 16S rDNA from uncultured sample

DNA from a wood sample (0.4 g) was extracted with the Bacteria DNA Preparation Kit (Jena Bioscience) according to manufacturer's recommendations. The sample was crushed before DNA extraction. The PCR mixture (35 μL) contained 1× Green GoTaq® Flexi Buffer (Promega), 0.2-mmol/L each deoxyribonucleoside triphosphate, 2 mmol/L $MgCl_2$, 1 μmol/L of each primer 27F- AGA GTT TGA TCM TGG CTC AG (Lane 1991) and 1407R- GAC GGG CGG TGW GTR CA (Lane et al. 1988), and 1.25 U of GoTaq® DNA Polymerase (Promega). The PCR amplification was performed at 95 °C for 5 min, 35 cycles, with each cycle consisting in 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 90 s, and a final extension at 72 °C for 10 min. A negative control without DNA template was amplified in the same conditions. The PCR mixes were loaded into an agarose gel (1 %) in TAE buffer (40 mmol/L Tris–acetate, 1 mmol/L EDTA, pH 8.0) with ethidium bromide (0.5 μg/mL). PCR products (~1,400 bp) were excised from the agarose gel and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega).

16S rDNA cloning

The 16S rDNA pool amplified by PCR from uncultured sample was cloned using the CloneJET™ PCR Cloning Kit (Thermo Scientific) according to manufacturer's recommendations. The ligation mixture was introduced by transformation into *Escherichia coli* cells strain XL1 blue (Chung and Miller 1993). About 96 colonies were randomly picked and

cultured in 5 mL of LB media for plasmid DNA extraction. The plasmid DNA was sequenced with vector sequencing primers (pJET1.2F and pJET1.2R).

Molecular identification of the cultured bacteria

In order to analyze the cultivable bacteria, colony PCR was performed under the same conditions described for the uncultured sample. The PCR product was purified from an agarose gel. Sequencing was done via a custom sequencing service (Macrogen Company, Amsterdam). The PCR products from cultured bacteria were sequenced with the 27F primer.

Identification of fungi

Genomic DNA was extracted from isolated colonies using the Animal and Fungi DNA Preparation Kit (Jena, Bioscience) according to manufacturer's instructions. PCR reactions were done with universal fungal primers ITS3 and ITS4 (White et al. 1990). PCR products (330 bp) were excised from the agarose gel and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). The DNA fragments were sequenced using the same primers used for PCR amplification.

Sequencing and sequence analysis

The 16S rDNA gene sequences were manually checked using the free analyzing software BioEdit version 7.0.9 (Tom Hall, Ibis Biosciences). The sequences were compared with the National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) using BLASTN (basic local alignment search tool) to retrieve similar sequences.

Results

Uncultivable microbial community

A total DNA extraction method was performed for a wood sample from the Nicula church. From the 16S rDNA library, 96 clones were sequenced. Analysis of the data obtained for these samples revealed the existence of *Bacillus* species (Table 1). Among the sequences, the predominant species were *Bacillus thuringiensis* (47 %) and *Solibacillus silvestris* (20 %). About 15 % of the clones represented less that 98 % identity when compare with sequences from NCBI nucleotide databases. At genus level, they are close to the *Bacillus* and *Solibacillus* genera. One percent of the clones showed 98 % similarity with *Paenibacillus amylolyticus* and 5 % with *Paenisporosarcina quisquiliarum*.

Cultivable bacteria

The cultivable bacteria from five icons were identified using colony PCR using 16S rDNA. A total of 66 colonies were analyzed (colonies were chosen by colony morphology). From these analyzed colonies, a total of 35 unique strains were identified using partial 16S rDNA sequence analysis (Table 2). The analysis of cultivated bacteria indicated also the predominance of Firmicutes, *Bacillus* genus. A number of 11 unique sequences belonged to Actinobacteria and 8 to Proteobacteria. The most common species found using both

Table 1 Bacteria obtained from uncultivated sample

Species	Accession number	Similarity (%)	% of total sequences
[Brevibacterium] frigoritolerans strain DSM 8801	NR_042639	99	1.1
Bacillus nealsonii strain DSM 15077	NR_044546	99	2.3
Bacillus soli strain R-16300	NR_025591	99	1.1
Bacillus thioparans strain BMP-1	NR_043762	99	3.4
Bacillus thuringiensis strain IAM 12077	NR_043403	99–100	47.2
Paenibacillus amylolyticus strain NRRL NRS-290	NR_025882	98	1.1
Paenisporosarcina quisquiliarum strain SK 55	NR_043720	99	5.6
Solibacillus silvestris strain HR3-23	NR_028865	98–100	20.2
Sphingomonas suberifaciens strain IFO 15211	NR_043394	99	1.1
Solibacillus		96–97	2.3
Bacillus sp.		92–97	14.6

Table 2 The diversity of cultivated bacteria

Domain	Phylum	Class	Order	Family	Species	Similarity (%)	Accession number
Bacteria	Actinobacteria	Actinobacteria	*Actinomycetales*	*Streptomycetaceae*	*Streptomyces exfoliatus* strain NBRC 13475	99	NR_041229
				Sanguibacteraceae	*Sanguibacter inulinus* strain ST50	99	NR_029277
				Dermabacteraceae	*Brachybacterium paraconglomeratum* strain LMG 19861	99	NR_025502
				Microbacteriaceae	*Frigoribacterium faeni* strain 801	99	NR_026511.1
					Microbacterium schleiferi strain DSM 20489	99	NR_044936
					Curtobacterium flaccumfaciens pv. *flaccumfaciens* strain LMG 3645	100	NR_025467
					Curtobacterium citreum strain DSM 20528	100	NR_026156.1
				Micrococcaceae	*Micrococcus luteus* strain DSM 20030	99	NR_037113
					Arthrobacter agilis strain DSM 20550	100	NR_026198
					Arthrobacter citreus strain DSM 20133	99	NR_026188
					Kocuria rosea	99	NR_044871
	Firmicutes	Bacilli	*Bacillales*	*Paenibacillaceae*	*Paenibacillus xylanilyticus* strain XIL14	98	NR_029109
				Planococcaceae	*Planomicrobium okeanokoites* strain IFO 12536	98	NR_025864
				Staphylococcaceae	*Staphylococcus cohnii* subsp. *cohnii* strain GH 137	100	NR_036902
					Staphylococcus equorum subsp. *equorum* strain PA 231	99	NR_027520
					Staphylococcus hominis subsp. *novobiosepticus* strain GTC 1228	99	NR_041323
				Bacillaceae	*[Brevibacterium] frigoritolerans* strain DSM 8801	99	NR_042639.1
					Bacillus amyloliquefaciens strain NBRC 15535	99	NR_041455
					Bacillus drentensis strain IDA1967	99	NR_029002
					Bacillus firmus strain IAM 12464	99	NR_025842
					Bacillus gibsonii strain DSM 8722	99	NR_026143
					Bacillus idriensis strain SMC 4352-2	100	NR_043268
					Bacillus lehensis strain MLB2	99	NR_036940
					Bacillus megaterium strain IAM 13418	99	NR_043401
					Bacillus niacini strain IFO 15566	99	NR_024695

Table 2 (continued)

Domain	Phylum	Class	Order	Family	Species	Similarity (%)	Accession number
					Bacillus sonorensis strain NRRLB-23154	99	NR_025130.1
					Bacillus safensis strain FO-036b	100	NR_041794
	Proteobacteria	Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Variovorax boronicumulans* strain BAM-48	98	NR_041588
		Alphaproteobacteria	*Rhodobacterales*	*Rhodobacteraceae*	*Paracoccus marcusii*	100	NR_044922
			Rhizobiales	*Rhizobiaceae*	*Rhizobium huautlense* strain SO2	97	NR_024863
			Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas aerolata* strain NW12	99	NR_042130
					Sphingomonas faeni strain MA-olki	99	NR_042129
		Gammaproteobacteria	*Pseudomonadales*	*Moraxellaceae*	*Psychrobacter alimentarius* strain JG-100	99	NR_025798
				Pseudomonadaceae	*Pseudomonas xanthomarina*	99	NR_041044
			Enterobacteriales	*Enterobacteriaceae*	*Pantoea dispersa* strain LMG 2603	98	NR_043883

approaches (cultivated and uncultivated) are *Brevibacterium frigoritolerans* and *P. quisquiliarum*.

Fungi associated with bacterial community

Fungal isolates were recovered from walls and icons. Based on morphology, about 38 colonies were selected for identification. From these samples, seven different fungi genera were identified on wooden walls and icon surfaces (Table 3). The *Alternaria*, *Penicillium*, and *Aspergillus* genera were identified on wall and icon surfaces. The *Cladosporium*, *Coprinellus*, and *Chaetomium* genera were identified only on the walls. The *Rhodotorula slooffiae* was identified only on icons.

Discussions

From uncultivated sample, the sequence analysis of the 16S rDNA library from a wood sample showed the prevalence of the *B. thuringiensis* strain IAM 12077, *S. silvestris* strain HR3-23 and *P. quisquiliarum* strain SK 55. From cultivated

Table 3 The diversity of fungi

Species	Similarity (%)	Accession number	Source
Alternaria alternata	100	KC292360	Icons, walls
Alternaria arborescens	100	KC415805	Walls
Aspergillus niveoglaucus	100	KC009789	Icons, walls
Aspergillus ruber	100	KC009779	Icons, walls
Cladosporium cladosporioides	100	KC865299	Walls
Coprinellus xanthothrix	100	HF543673	Walls
Chaetomium subglobosum	100	JN209930	Walls
Penicillium citreonigrum	100	JN689966	Icons, walls
Penicillium verrucosum	100	KC009832	Walls
Penicillium glabrum	100	KC797645	Walls
Rhodotodula slooffiae	100	JQ993376	Icons
Fungal endophyte sp. ECD-2008	99	EU686200	Walls
Uncultured soil fungus	90	EU479954	Walls

bacteria, the presence of *Planomicrobium* and *Variovorax* genera was not previously reported in paintings or on wood; the strains are most probably of environmental origin. The fungal genera *Alternaria*, *Penicillium*, and *Aspergillus* were found on church walls and icons.

The *Bacillus* is a diverse genus, including hundreds of strains (Porwal et al. 2009; Capodicasa et al. 2010). This predominance can be explained by its prevalence in environmental samples and its ability to survive in harsh conditions (Fajardo-Cavazos and Nicholson 2006; Osman et al. 2008) by forming endospores. Another explanation of the prevalence of *Bacillus* in wood could be the presence in its genome of genes encoding cellulose degrading enzymes or laccase-like enzymes (Khalid et al. 2012). *B. thuringiensis* is Gram-positive spore-forming bacteria that can produce cellulases, which can mediate the breakdown of cellulose (Lin 2012). *Sphingomonas suberifaciens* is a soil bacterium that can cause a corky root disease in lettuce, indicating that it may have some lignin-degrading enzymes (Krishnamurthi et al. 2009). *P. amylolyticus* identified in the sample is known for its capacity to degrade biomasses, especially those containing pectin, due to the pectate lyase enzymes (Boland et al. 2010). The presence and the predominance of *Bacillus* strains were revealed by both the culture-independent and culture-dependent approaches. Most of the isolated bacteria are of environmental origin. Among these species are bacteria known to be present on wall paintings, such as *Arthrobacter*, *Bacillus*, *Paenibacillus*, *Staphylococcus*, *Pseudomonas*, *Micrococcus* (Pepe et al. 2010), *Sphingomonas* (Seves et al. 1996), and *Curtobacterium* of rock paintings (Urzì et al. 2010). *Paenibacillus xylanyliticus* is known as a xylan-degrading bacterium that can use xylan as a unique source of carbon (Rivas et al. 2005). *Bacillus* genus was also found present on paintings (Capodicasa et al. 2010; Pepe et al. 2010), mural paintings (Heyrman 2003), and frescoes (Radaelli et al. 2004).

Human skin microbiota is very abundant in Proteobacteria species and Firmicutes represented by the *Staphylococcaceae* family (Grice and Segre 2011). The presence of *Staphylococcus cohnii*, *Staphylococcus hominis*, and *Pantoea* on icons is of human microbiome origin. These species can have high methicillin resistance and resistance to other antimicrobials, posing problems in interpersonal exchanges and also in the transfer of antibiotic resistance genes to other species (Garza-González et al. 2011), but are not involved in wood degradation. The *Micrococcus* is very abundant in air, as many as two thirds of the airborne bacteria being micrococci (Kooken et al. 2012), and contributes to the interpersonal exchange of different strains. The genera *Streptomyces*, *Paenibacillus*, and *Bacillus* were found in bacterial communities on wooden art objects and air microflora and have revealed cellulolytic activity (Pangallo et al. 2007). In a more recent study establishing the characteristics of microbial communities isolated from indoor artworks, the same author showed the presence of predominance of *Bacillus* and *Staphylococcus* genera with biodegradation properties (Pangallo et al. 2009).

Fungi are considered the most important microorganisms contributing to the biodegradation of the wood samples due to their cellulolytic and proteolytic activities. The presence of fungi is important not only for wood degradation but also for painting degradation. The presence of *Alternaria*, *Aspergillus*, *Penicillium*, and *Cladosporium* genera identified in the present study are always predominant in indoor and outdoor atmospheres (Rivas et al. 2005). According to Ciferri (1999), the bacteria of the *Bacillus* genus cannot grow on paintings by themselves, but fungi as *Aspergillus* and *Penicillium* promote the further survival of bacteria on paintings. It has been proposed that fungi are first colonizers of the paintings, and the wood and their metabolic products are used by bacteria (Capodicasa et al. 2010). Fungi like *Alternaria*, *Aspergillus*, and *Cladosporium* genera are highly abundant in air. It is estimated that emission rate per person-hour in indoor airborne is 7.3×10^6 fungi genome copies for total particle mass (Qian et al. 2012).

To the best of our knowledge, this is the first study which did a microbiological evaluation of the wooden churches from this part of Europe. Our study will contribute to a better understanding of the new and old microbial community involved in wood biodegradation and to establishing preservation strategies of wood monuments.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Boland WE, Henriksen ED, Doran-Peterson J (2010) Characterization of two *Paenibacillus amylolyticus* strain 27C64 pectate lyases with activity on highly methylated pectin. Appl Environ Microbiol 76:6006–6009. doi:10.1128/AEM.00043-10

Capodicasa S, Fedi S, Porcelli AM, Zannoni D (2010) The microbial community dwelling on a biodeteriorated 16th century painting. Int Biodeter Biodegrad 64:727–733. doi:10.1016/j.ibiod.2010.08.006

Chung CT, Miller RH (1993) Preparation and storage of competent *Escherichia coli* cells. Methods Enzymol 218:621–627

Ciferri O (1999) Microbial degradation of paintings. Appl Environ Microbiol 65:879–885

Fajardo-Cavazos P, Nicholson W (2006) *Bacillus* endospores isolated from granite: close molecular relationships to globally distributed *Bacillus* spp. from endolithic and extreme environments. Appl Environ Microbiol 72:2856–2863. doi:10.1128/AEM.72.4.2856

Garza-González E, Morfin-Otero R, Martínez-Vázquez MA, Gonzalez-Diaz E, González-Santiago O, Rodríguez-Noriega E (2011) Microbiological and molecular characterization of human clinical isolates of *Staphylococcus cohnii*, *Staphylococcus hominis*, and *Staphylococcus sciuri*. Scand J Infect Dis 43:930–936. doi:10.3109/00365548.2011.598873

Gaylarde CC, Morton LHG, Loh K, Shirakawa MA (2011) Biodeterioration of external architectural paint films—a review. Int Biodeter Biodegrad 65:1189–1198. doi:10.1016/j.ibiod.2011.09.005

Grice EA, Segre JA (2011) The skin microbiome. Nat Rev Microbiol 9:244–253. doi:10.1038/nrmicro2537

Heyrman J (2003) *Bacillus decolorationis* sp. nov., isolated from biodeteriorated parts of the mural paintings at the Servilia tomb (Roman necropolis of Carmona, Spain) and the Saint-Catherine chapel (Castle Herberstein, Austria). Int J Syst Evol Microbiol 53:459–463. doi:10.1099/ijs.0.02452-0

Imperi F, Caneva G, Cancellieri L, Ricci MA, Sodo A, Visca P (2007) The bacterial aetiology of rosy discoloration of ancient wall paintings. Environ Microbiol 9:2894–2902. doi:10.1111/j.1462-2920.2007.01393.x

Jurado V, Ortiz-Martinez A, Gonzalez-delValle M, Hermosin B, Saiz-Jimenez C (2002) Holy water fonts are reservoirs of pathogenic bacteria. Environ Microbiol 4:617–620. doi:10.1046/j.1462-2920.2002.00341.x

Khalid A, Kausar F, Arshad M, Mahmood T, Ahmed I (2012) Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment. Appl Microbiol Biotechnol 96(6):1599–1606. doi:10.1007/s00253-012-3877-7

Kooken JM, Fox KF, Fox A (2012) Characterization of *Micrococcus* strains isolated from indoor air. Mol Cell Probes 26:1–5. doi:10.1016/j.mcp.2011.09.003

Krishnamurthi S, Bhattacharya A, Mayilraj S, Saha P, Schumann P, Chakrabarti T (2009) Description of *Paenisporosarcina quisquiliarum* gen. nov., sp. nov., and reclassification of *Sporosarcina macmurdoensis* Reddy et al. 2003 as *Paenisporosarcina macmurdoensis* comb. nov. Int J Syst Evol Microbiol 59:1364–1370. doi:10.1099/ijs.0.65130-0

Lane D (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, Chichester, pp 115–175

Lane DJ, Field KG, Olsen GJ, Pace NR (1988) Reverse transcriptase sequencing of ribosomal RNA for phylogenetic analysis. Methods Enzymol 167:138–144

Lin L (2012) Characterization of extracellular cellulose-degrading enzymes from *Bacillus thuringiensis* strains. Electron J Biotechnol 15:1–7. doi:10.2225/vol15-issue3-fulltext-1

Osman S, Peeters Z, La Duc MT, Mancinelli R, Ehrenfreund P, Venkateswaran K (2008) Effect of shadowing on survival of bacteria under conditions simulating the Martian atmosphere and UV radiation. Appl Environ Microbiol 74:959–970. doi:10.1128/AEM.01973-07

Pangallo D, Simonovicova A, Chovanova K, Ferianc P (2007) Wooden art objects and the museum environment: identification and biodegradative characteristics of isolated microflora. Lett Appl Microbiol 45:87–94. doi:10.1111/j.1472-765X.2007.02138.x

Pangallo D, Chovanova K, Simonovicova A, Ferianc P (2009) Investigation of microbial community isolated from indoor artworks and air environment: identification, biodegradative abilities, and DNA typing. Can J Microbiol 55:277–287. doi:10.1139/w08-136

Pepe O, Sannino L, Palomba S, Anastasio M, Blaiotta G, Villani F, Moschetti G (2010) Heterotrophic microorganisms in deteriorated medieval wall paintings in southern Italian churches. Microbiol Res 165:21–32. doi:10.1016/j.micres.2008.03.005

Piñar G, Ramos C, Rölleke S et al (2001) Detection of indigenous *Halobacillus* populations in damaged ancient wall paintings and building materials: molecular monitoring and cultivation. Appl Environ Microbiol 67:4891–4895. doi:10.1128/AEM.67.10.4891

Piñar G, Ripka K, Weber J, Sterflinger K (2009) The micro-biota of a sub-surface monument the medieval chapel of St. Virgil (Vienna, Austria). Int Biodeter Biodegrad 63:851–859. doi:10.1016/j.ibiod.2009.02.004

Portillo MC, Gonzalez JM, Saiz-Jimenez C (2008) Metabolically active microbial communities of yellow and grey colonizations on the walls of Altamira Cave, Spain. J Appl Microbiol 104:681–691. doi:10.1111/j.1365-2672.2007.03594.x

Portillo MC, Alloza R, Gonzalez JM (2009) Three different phototrophic microbial communities colonizing a single natural shelter containing prehistoric paintings. Sci Total Environ 407:4876–4881. doi:10.1016/j.scitotenv.2009.05.038

Porwal S, Lal S, Cheema S, Kalia VC (2009) Phylogeny in aid of the present and novel microbial lineages: diversity in *Bacillus*. PLoS One 4:e4438. doi:10.1371/journal.pone.0004438

Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J (2012) Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. Indoor Air 22:339–351. doi:10.1111/j.1600-0668.2012.00769.x

Radaelli A, Paganini M, Basavecchia V, Elli V, Neri M, Zanotto C, Pontieri E, De Giuli Morgen C (2004) Identification, molecular biotyping and ultrastructural studies of bacterial communities isolated from two damaged frescoes of St Damian's Monastery in Assisi. Lett Appl Microbiol 38:447–453. doi:10.1111/j.1472-765X.2004.01514.x

Rivas R, Mateos PF, Martínez-Molina E, Velázquez E (2005) *Paenibacillus xylanilyticus* sp. nov., an airborne xylanolytic bacterium. Int J Syst Evol Microbiol 55:405–408. doi:10.1099/ijs.0.63173-0

Rölleke S, Muyzer G, Wawer C, Wanner G, Lubitz W (1996) Identification of bacteria in a biodegraded wall painting by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl Environ Microbiol 62:2059–2065

Seves AM, Sora S, Ciferri O (1996) The microbial colonization of oil paintings. A laboratory investigation. Int Biodeter Biodegrad 37:215–224. doi:10.1016/S0964-8305(96)00006-6

Sterflinger K, Pinzari F (2012) The revenge of time: fungal deterioration of cultural heritage with particular reference to books, paper and parchment. Environ Microbiol 14:559–566. doi:10.1111/j.1462-2920.2011.02584.x

Urzì C, De Leo F, Bruno L, Albertano P (2010) Microbial diversity in paleolithic caves: a study case on the phototrophic biofilms of the Cave of Bats (Zuheros, Spain). Microb Ecol 60:116–129. doi:10.1007/s00248-010-9710-x

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322