

Biodeterioration of Gold medieval fresco fragments painted at Holy Nail’s Chapel, SIENA, Italy

Claudio Milanesia*, Franco Baldi^b, Sara Borini^c, Lorenzo Brusetti^c, Fabrizio Ciampolinia^a, Mauro Cresti^a

- a) Department of Environmental Science ‘G. Sarfatti’, University of Siena, P.A. Mattioli 4, 53100, Siena, Italy, *Corresponding author, e-mail: milanesi@unisi.it
b) Department of Environmental Science Cà Foscari University of Venezia, Calle Larga S. Marta, 30121, Venezia, Italy.
c) Department of Food Science and Technology, University of Milan, Celoria 2, 20133 Milan Italy.

Biodeterioration of paintings is a field of multidisciplinary interest that draws from the arts, humanities and many branches of science. Medieval masterpieces combine colors having different, often toxic, mineral compositions (Cennini, C., 1390 ca. Il libro dell’arte, ed. 1943, Marzocco, Firenze, Italy), containing metals such as arsenic, mercury, lead and cadmium (Ciferri, 1999). Recent restoration of the Chapel of the Holy Nail in Siena Italy, (Fig. 1) was a good opportunity to study the deteriorated part of a fresco by Lorenzo di Pietro who is known as “il Vecchietta” (Vasari, G., 1555). It was possible to obtain tiny fragments of fresco (Fig. 1 arrow). Samples were placed in sterile plastic tubes and their composition was analysed by scanning microscopy with x-ray dispersion.

Elemental analysis revealed carbon (14.01%), sulfur (11.05%), calcium (23.86%) and other less representative elements (Fig. 2). The painting contained the inorganic pigment *orpimento* (As₂S₃), a cheap substitute for gold, mixed with animal glue (Cennini, C., p. 47), probably collagen and fatty acids. Iron and copper were not found and indeed were avoided because they caused blackening of the *orpimento*. In the present case, blackening was due to environmental humidity. Environmental humidity also caused production of sulfates on the surface, facilitating degradation of the *orpimento* through volatilization of arsenic to form methylated arsenic (methylarsine), presumably by deteriogens.

Since there was no light microscope evidence of fungal hyphae, probably due to neutral pH and the absence of iron and copper (Milanesi et al. 2006), we surveyed microbial community diversity by 16S rRNA gene clone libraries. Two libraries were made: one from an original fresco sample and a second from a fresco fragment placed in mineral medium without any carbon source, in order to cultivate quiescent bacteria able to grow on endogenous carbon sources in the fragment.

Only two genera (*Bacillus* sp. and *Brevibacillus* sp.), both belonging to the Firmicutes, were found in the latter sample, whereas great biodiversity was found in the original fresco sample: 95 clones of bacteria belonging to seven different taxa (Fig 3). The largest groups were Firmicutes (44%), beta and alpha Proteobacteria (17%), Actinobacteria (9%), gamma-Proteobacteria (8%), Bacteroidetes (4%) and Fusobacteria (1%). Firmicutes of the genera *Bacillus* and *Brevibacillus* were only occasionally found, most of the Firmicutes belonging to the genus *Streptococcus*.

Excluding unknown bacteria (27%), most of the others originated from human beings and are associated with human diseases (Fig. 4). This is not surprising because the Chapel of the Holy Nail is inside *Spedale Santa Maria della Scala*, a hospital, founded in 1100, where pilgrims were cared for. This, however, is the first report of human-associated bacteria actively living on frescoes and indicates the close relationship between biodeteriorating bacterial communities and the history of the site where the frescoes were painted.



Figure 1. Holy Nail’s Chapel, Museum complex Santa Maria della Scala, Italy, before restore.



Figure 1. Holy Nail’s Chapel at Siena (Italy), showing hold testament and gold painting change in black color (arrow)

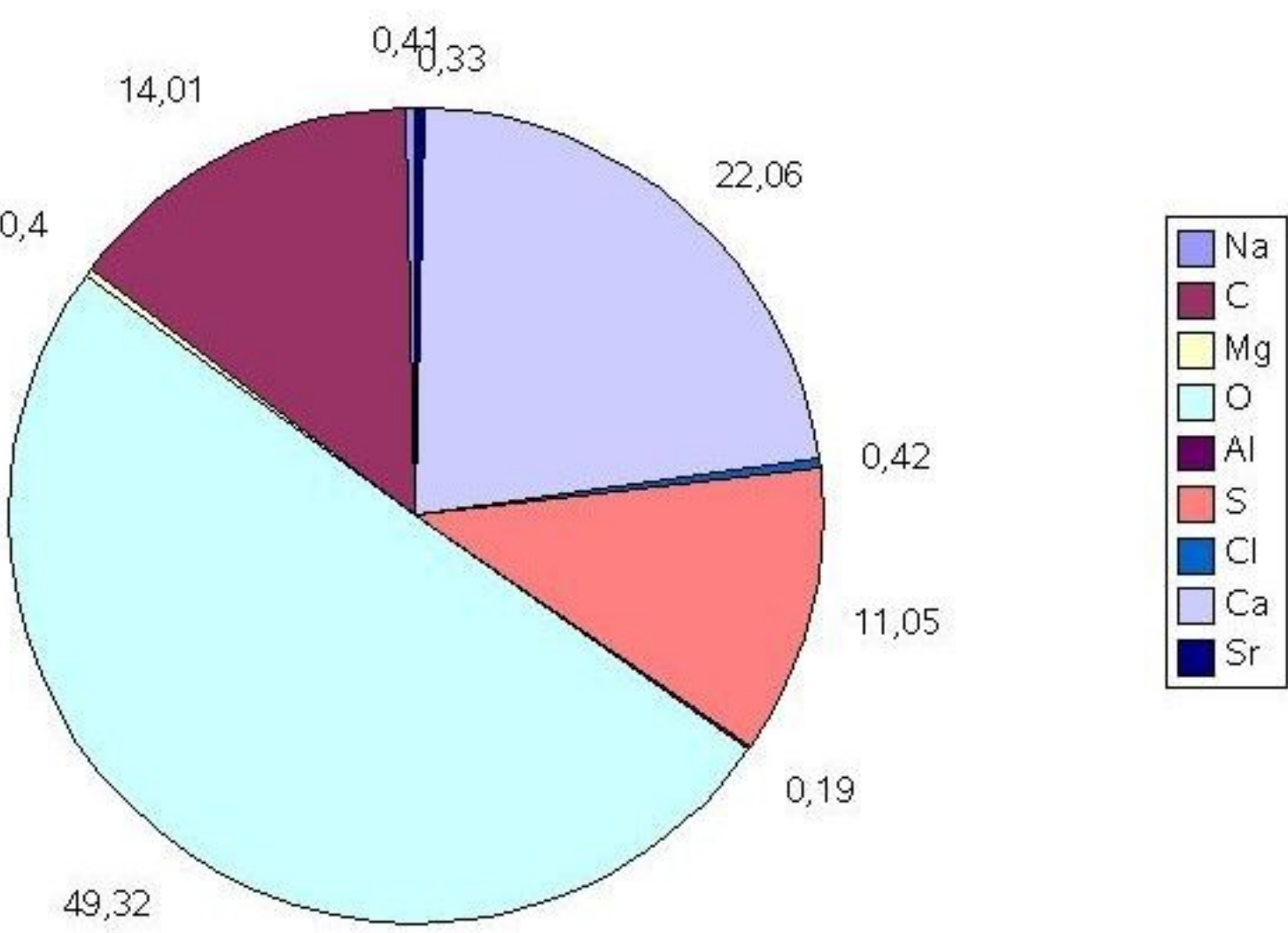


Figure 2. Microanalyses of elements on the original fragments by SEM equipped with energy dispersive x-ray system (EDAX-DX4)

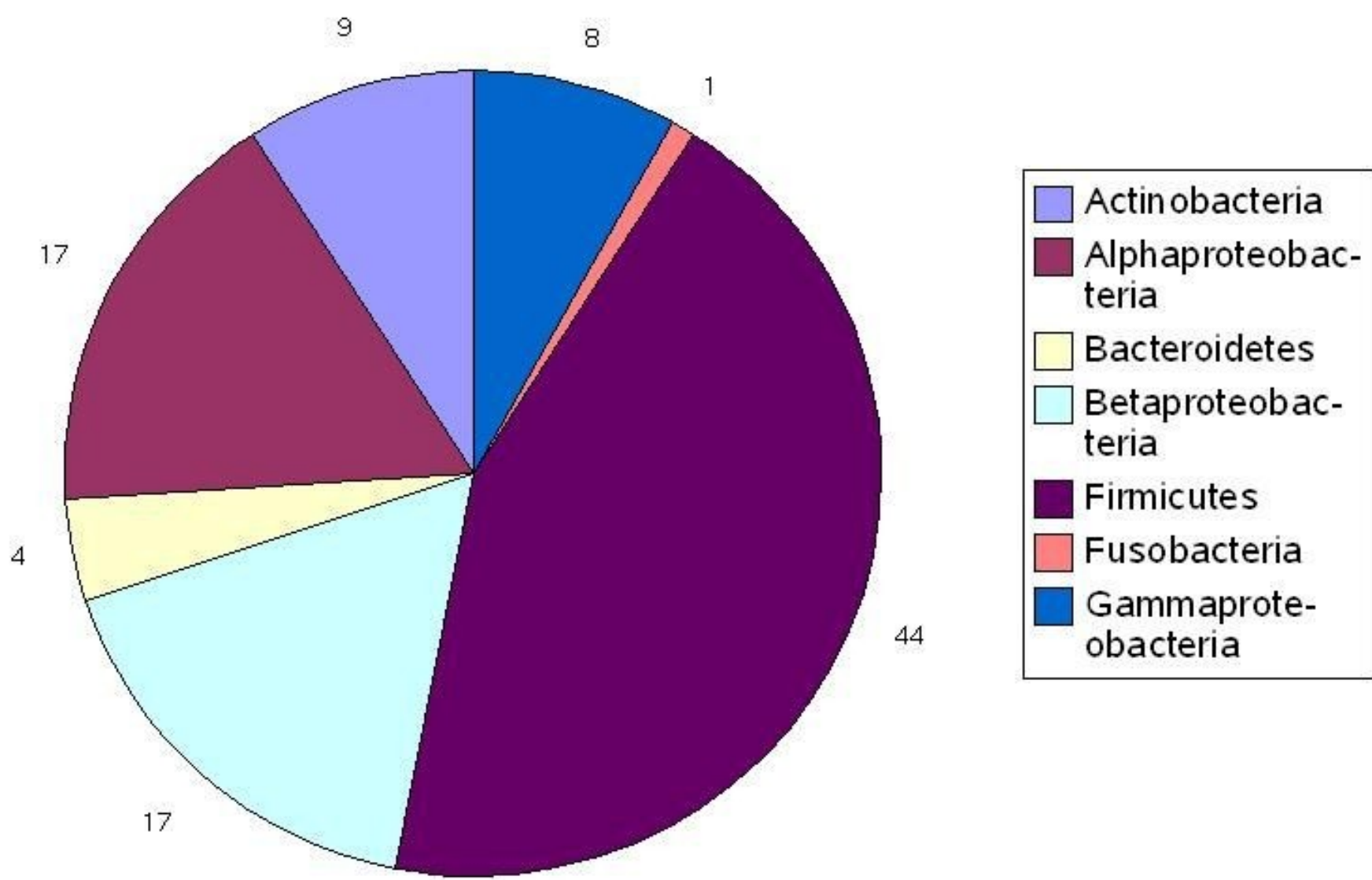


Figure 3. Biodiversity in the original fresco fragments: 95 clones bacteria to seven different taxa.

Sampling of fresco obtaining, hydrated and incubated

The history chapel of Sacro Chiodo church situated in Santa maria della Scala Museum Siena (Italy) were described in Milanesi et al. 2006. Specimens (approximately 1 x 3 mm and 0.5 mm thick) were obtained in the gold oxidations areas (Fig. 1 arrow). The irregular fragments were detached from the fresco using sterile tweezers and placed in sterile plastic tubes and numbered. Two irregular fragment were incubated in solid medium containing per liter: 1 g MgSO₄·7H₂O, 0.7 g KCl, 2 g KH₂PO₄, 3 g Na₂HPO₄, 1 g NH₄NO₃, at pH 6.7.

Scanning Microscope observations and microanalysis

Original fragment directly detached from mural painting were glued to standard vacuum-clean stubs and coated with graphite (Edwards, carbon scancoat, S150A) and observed by SEM (Philips XL20). The instrument was equipped with an EDAX DX4 probe for energy-dispersive x-ray microanalysis used at 20 kV acceleration voltage for determining characteristic of the different of pictorial surface. Concentrations had an approximate error of 1%. Mean concentrations and standards deviations of each element were calculated from five random determinations on different spots of analysed sample. The X ray beam was 4 µm wide and penetrated to a depth of 2 µm.

Isolation of microorganisms

Culture was visibly negative for fungal life after 18 h. This was confirmed by light microscopy observation. Incubated fragments, were transferred with sterile tweezers for identified by sequencing the 16S rRNA gene.

Identification of bacteria, DNA amplification and sequencing

To identify and compared quiescent microorganism involved in fresco dry fragment deterioration, and from incubated wall fragment strains were DNA extraction, amplifications and sequencing by 16S rRNA gene. Total DNA was extracted and purified using FastDNA™ SPIN Kit for Soil (BIO 101 Systems Q-BIO gene; CA, USA) following the manufacturer’s instructions. DNA quantity and integrity was checked by agarose electrophoresis and comparison with Lambda phage DNA of known amounts, and by spectrophotometrically measuring the OD₆₀₀. The almost complete bacterial 16S rRNA genes were amplified from the total DNA extracted from sediments, using universal primers for bacteria 27f and 1440r, in a 100 µl reaction mixture containing 0.5 µM of each primer, 0.12 mM of dNTPs mixture, 1.5 mM MgCl₂, 0.5 U of Taq polymerase with the provided 1× buffer (Invitrogen, Milan, Italy) and 100 ng of DNA template. The thermal protocol was: initial denaturation 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and a final elongation at 72°C for 10 min. The amplicons were purified using the Qiaquick PCR purification kit (Qiagen, Milan, Italy) following the instructions of the manufacturer. The purified fragments were ligated in the pGEMT vector and cloned in JM300 competent *E. coli* cells (Promega, Milan, Italy) following the instructions of the manufacturer. Positive clones were screened for insert presence by PCR using the universal primers T7 and U19 (Promega, Milan, Italy), and about 800 bp were sequenced (Primm, Milan, Italy), with the 27f primer, corresponding to the first half of the 16S rRNA gene. Sequences were checked using the CHECK_CHIMERA program to determine the presence of any hybrid sequences. Phylogenetic affiliations were obtained using BLASTN, and confirmed with the naïve Bayesian rRNA Classifier provided by the Ribosomal Database Project website.

Phylum	%	Genus [%of probability]
Actinobacteria	1	Corynebacterium[100%]
Actinobacteria	2	Kocuria[100%]
Actinobacteria	1	Propionibacterium[100%]
Actinobacteria	5	Rothia[100%]
Alphaproteobacteria	1	Asaia[100%]
Alphaproteobacteria	3	Bradyrhizobium[100%]
Alphaproteobacteria	6	Gluconobacter[100%]
Alphaproteobacteria	3	Sphingomonas[100%]
Alphaproteobacteria	3	Sphingopyxis[88%]
Bacteroidetes	1	Pedobacter[100%]
Bacteroidetes	3	Prevotella[100%]
Betaproteobacterium	9	Burkholderiales Incertae sedis [100%] (Ralstonia-like)
Betaproteobacterium	1	Burkholderiales Incertae sedis [99%] (Leptothrix-like)
Betaproteobacterium	2	Neisseriaceae[94%] (Vitreoscilla-like)
Betaproteobacterium	2	Neisseriaceae[98%] (Simonsiella-like)
Betaproteobacterium	1	Oxalobacteraceae[100%]
Betaproteobacterium	1	Ralstonia[100%]
Firmicutes	2	Abiotrophia[100%]
Firmicutes	2	Anaerococcus[100%]
Firmicutes	1	Bacillus[100%]
Firmicutes	1	Clostridium[100%]
Firmicutes	3	Dolosigranulum[100%]
Firmicutes	5	Granulicatella[100%]
Firmicutes	27	Streptococcus[100%]
Fusobacteria	1	Fusobacterium[100%]
Gammaproteobacteria	2	Gammaproteobacteria[96%] (Oceanospirillales-like)
Gammaproteobacteria	1	Haemophilus[95%]
Gammaproteobacteria	2	Moraxella[100%]
Gammaproteobacteria	2	Nevskia[100%]
Gammaproteobacteria	1	Xanthomonadaceae[100%] (Lysobacter-like)

Fig. 4. The largest group were Firmicutes of the genera *streptococcus* (27%), associated with human diseases.

Conclusion

In the Vecchietta Medieval fresco painting blackening was due to environmental humidity and facilitating degradation of the *orpimento* presumably by deteriogens. By light microscope was no evidence of fungal hyphae, probably due to neutral pH and the absence of iron and copper and we surveyed microbial community diversity by 16S rRNA gene clone libraries. Only two genera (*Bacillus* sp. and *Brevibacillus* sp.), both belonging to the Firmicutes were found in the latter samples. In the 95 clones of bacteria belonging to seven different taxa were found in the original fresco sample. The largest groups were Firmicutes (44%), most of the Firmicutes belonging to the genus *Streptococcus* associated with human diseases. The Chapel of the Holy Nail is started to X century inside a hospital and this indicates the relationship between biodeteriorating bacterial communities and the history of the site where the frescoes were painted.

Reference

Cennini, C., 1390 ca. Il libro dell’arte, ed. 1943, Marzocco, Firenze, Italy
Ciferri, O., 1999. Microbial degradation of paintings. Applied Environmental Microbiology 65, 879-885.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118.
Gurtner, C., Heyrman, J., Piñar, G., Lubitz, W., Swings, J., Rölleke, S., 2000. Comparative analyses of the bacterial diversity on two different biodeteriorated wall paintings by DGGE and 16S rDNA sequence analysis. International Biodeterioration & Biodegradation 46, 229-239.
Jasalavich, C.A., Ostrofsky, A., Jellison, J., 2000. detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA. Applied and Environmental Microbiology 66, 4725–4734.
Milanesi, C., Baldi, F., Vignani, R., Ciampolini, F., Faleri, C., Cresti, M., 2006. Fungal deterioration of Medieval wall fresco determined by analysing small fragments containing copper, International Biodeterioration& Biodegradation 57 (2006) 7–13
G. Vasari, Le vite de’ più eccellenti architetti, pittori et scultori italiani da Cimabue insino a’ tempi nostri, Firenze, Lorenzo Torrentino, 1550, ed. 1986, L. Bellosi e A. Rossi, Einaudi, Torino, Italy.