A strain grows on endogenous carbon of deteriorated fresco fragments (Santissima Annunziata, Siena) producing a biofilm on the surface

Claudio Milanesi, Franco Baldi*, Sara Borin**, Rita Vignani,
Fabrizio Ciampolini, Claudia Faleri and Mauro Cresti

Department of Environmental Science "G. Sarfatti", University of Siena, P.A. Mattioli 4, 53100, Siena, Italy.
*Department of Environmental Science, C Foscari University, Calle Larga S. Marta, 30121 Venezia, Italy
** Department of Food Science and Technology, University of Milan, Celoria 2, 20133 Milan Italy

Three areas (A,B,C) of the fresco in the apse of the church (Santissima Annunziata, Siena) showed signs of deterioration. Sebastiano Conca artist in the 1730, dilutes pigments with calcium carbonate and washes them over the base before it is completely dry. The tempera does not penetrate very deeply and the fresco surface remains rough. Humidity, poor ventilation and light (including artificial light) facilitate formation of biofilms composed of viscous EPS, which are hydrogels consisting of about 98% water, that adhere to the surface (Morton and Surman, 1994), increasing microbial concentration, and diversification and causing detachment of the rough paint layer (Ciferri, 1999). Fragments detached from tree different areas of the fresco were soaked in mineral medium without carbon source. No bacterial growth was evident for samples from areas B and C, after 72 h of incubation while two strains were isolated from area A and identified as Kocuria erythromyxa strain CV1 and Sphingomonas echinoides strain CV2 by sequencing gene 16S rDNA. Our hypothesis was that microorganisms CV1 and CV2 which damage fresco should grow on the carbon nutrients of the surface. SEM microanalysis observations of the original fragments confirmed this hypotheses and showed from area A high total carbon concentrations respect other two areas. Laser scanning confocal microscopy with Con-A fluorescent lectin and Ultrastructural analysis of sections of the microorganisms by transmission electron microscopy showed that only strain CV2 adhered to the substrate by mucilage consisted mainly of Extracellular Polymeric Substances and caused biological deterioration of the fresco.

Fig. 1. Eighteenth century fresco by Sebastiano Conca in the apse of the chapel of the Santissima Annunziata, Siena, showing damaged areas A, B and C where fragments were sampled for analyses.

C Sampling ws performed according to Italian legislative procedure (DL 3/1980). Specimens were obtained in three damage areas (A,B,C) of the 18th fresco by Sebastiano Conca in the apse of the nchurch Santissima Annunziata in Siena (Fig. 1). Two of each set of 12 fragments were prepared for element composition by SEM-EDX (Philips XL20). Specimens were glued and coated with grafite (Edwards, carbon cancoat, SI50A). The X-ray beam was 4μm wide and penetrated to a depth of 2μm. In order to enrich the bacterial population thriving on endogenous carbon as the sole carbon and energy source, each damaged area (measuring 20x20 cm) was wiped with sterile cotton swab. The sample corresponding to each damaged area was incubated at 28 C in test tubes in 10 ml of mineral medium containing per liter: 1 g MgSO4.7H2O, 0,7 g KCl, 2 g KH2PO4, 3g Na2HPO4, 1 g NH4NO3, at pH 6,7. Only the sample from site A was positive for bacterial growth. The mixed culture was transferred to nutrient agar broth (Difco) to isolate two strains identified as CV1 and CV2. The two strain were identified by sequencing the 16S rRNA gene and using Treecom program: The sequences obtained are available in GenBank under accession nos. DQ176452 (CV1) and DQ176453 (CV2). The fragment from area A incubated in mineral medium was completely coated bacteria. Image analysis of this specimens was performed by CLSM (BioRad model Microradiance MRAG1). The fluorescent molecular probe ConA (lectin Concavalin A) was used for its affinity for glucose and mannose redidues. In addition, in the incubated and non incubated fragments of damaged area A were prepared and than observed for TEM amalyses to determine ultrastructural interactions between bacteriua and the inorganic substrate of fresco.

B

A

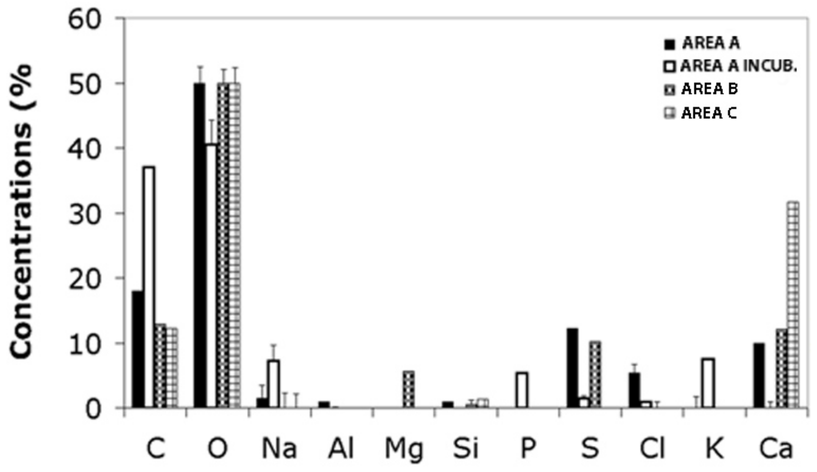

Fig. 2. SEM/EDAX X-ray microanalysis of elemental composition of fresco surface without incubation of pictorial fragments from areas A, B and C and a fragment from area A, which was incubated in mineral medium.

Fig. 3. SEM observation in original fresco fragments without incubations : (**A**) chemical deterioration of fresco surface showing saline efflorescence (E), (bar = 10 μm) in site C; (**B**) fresco surface with abundant saline efflorescence (E), (bar = 5 μm)in site B; (**C**) fresco surface with quiescent microbes (bar = 10 μm) in site A.

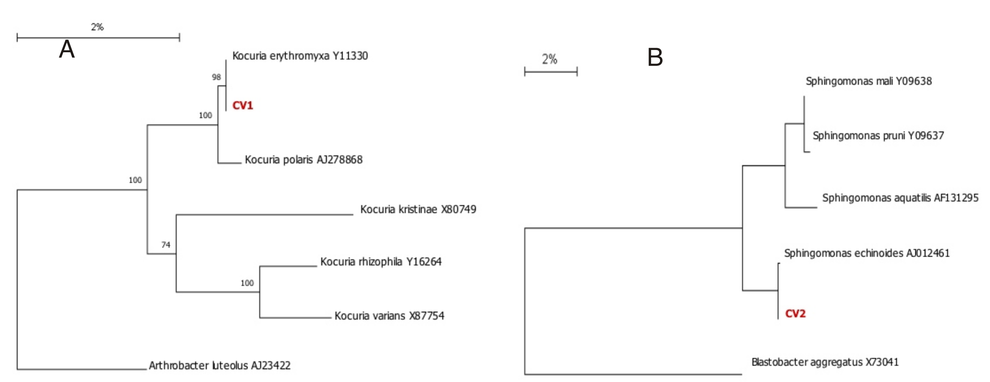

Fig. 4. A) Cladogram sequences aligned in public databases (GenBank). Strain CV1 (GenBank accession nos. D0176452) belonged to the order Actinomycetales and was identified as Kocuria erythromyxa (ex Micrococcus roseus) with 100% sequence homology with the reference strain. **B)** Strain CV2 (Gen Bank accession nos. D0176453) belonged to the Sphingomonadales and was identified as Sphingomonas echinoides (ex Pseudomonas echinoides) with 100% sequence homology**.**

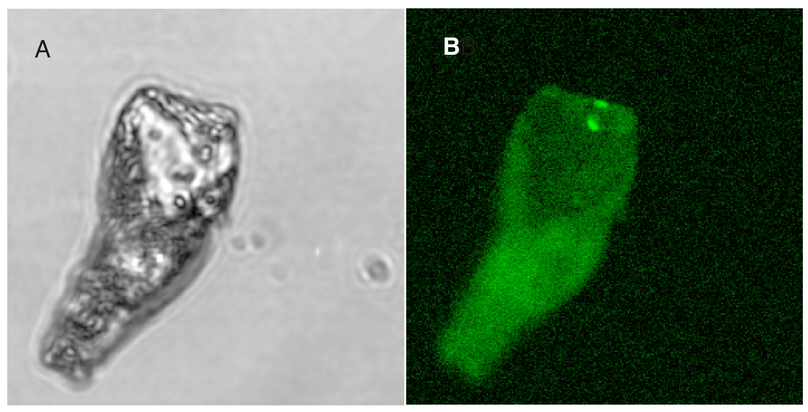

Fig. 5. (**A**) Image in trasmissiom mode of a microfragment of pictorial patina incubated in mineral medium, which is colonized by pointed rod elements of S. echinoides strain CV2 adhering to the surface of a sample; (**B**) Laser scanning confocal micrograph of specimens stained with ConA-FITC fluorescence of extracellular polysaccharides containing glucose and mannose and covering much of the surface of the microfragment (x630).

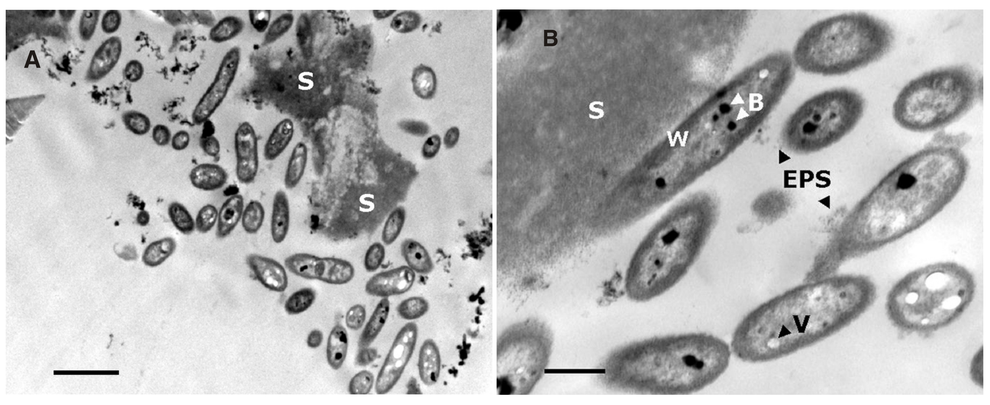

Fig. 6. **A**) TEM micrograph showing adhesion of strain S. echinoides CV2 to the fresco substrate (**S**). The strain grew in mineral medium with endogenous carbon source from sampling area (bar = 1,4 micron). **B**) Transmission electron micrograph showing a detailed fresco substrate (**S**), cell wall (**W**), cytoplasm with vacuole (**V**), black bodies in cytoplasm and extracellular polysaccahride (**EPS**), (bar = 0.70 μm).

Conclusions

A major difficulty of this study was the small weight of samples. In situ techniques were therefore useful, because they only require small amounts of material. Areas A and B showed the highest total sulphur concentrations. Site A was the most damaged and had the highest total carbon content. The mixture of carbon compounds, originating from human and/or previous microbial activity, supported microbial growth of Sphingomonas and Kokuria strains. S. echinoides CV2 produced a moisture-retaining biofilm on the fresco surface. EPS of the biofilm may stimulate the growth of other microorganisms, increasing the damage. The deterioration in area A could therefore have been caused by rising damp from the floor combined with microbial activity.

.

References

Ciferri, O., 1999. Microbial degradation of paintings. Applied Environmental Microbiology 65, 879-885.

Morton, L. H. G., Surman, S. B., 1994. Biofilms in biodeterioration. International Biodeterioration & Biodegradation 34, 203-221