

## Biodegradation assessment of a 16<sup>th</sup> century fresco from Southern Portugal

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This work reports the study of the *frescoes* from the *Casa de Fresco dos Sanches Baena* in Vila Viçosa (Southeast Portugal) to allow their material characterisation, to identify the different microorganism populations and to assess their role in the deterioration of these paintings.

The *Casa de Fresco dos Sanches Baena* is a small semi-underground building constructed in a Palace garden over a well and used as a cool refreshing place by the owners. The frescoes that cover the ceilings (composed of 4 panels) present rich mythological scenes and have other decorative elements, which make them an unusual example of this art form. Unfortunately, due to partial abandonment the paintings are in an advanced state of degradation exhibiting partial detachment of paint layers and mortars, salt efflorescences and abundant biological colonisations (Fig.1).

Microsampling of paint layers was performed on representative areas of the paintings. The cross-sections were analysed by optical microscopy, microchemical analysis and scanning electron microscopy coupled with energy dispersive spectrometry allowing pigment identification and stratigraphy analysis. The results showed that azurite and malachite were used for the blues and greens, respectively. As for the reds and yellows, the results showed that ochres were used.

For the microbiologic sampling, sterile cotton buds and chisels were used and the biological materials collected in sterile recipients. Colonies morphologies were evaluated by microscope preparations. Gram's stains and some usually tests followed by *Bergey's Manual of Systematic Bacteriology* [1] were used for bacteria identification. Fungal strains were identified following standard methods [2], based on its macro and micro-morphological characteristics, such as colony diameter, texture, colour, dimensions and morphology of hyphae and reproductive structures (for sporulating isolates). The microbiological study (fig. 2 and 3) by optical microscopy allowed the isolation of 34 fungi strains and 32 bacterial strains in the four painted panels that compose the frescoes. Panel 2 presented the highest microbial contamination with 19 fungi strains and 18 bacterial strains observed. The predominant bacterial strains were bacillus Gram+ and Gram-, from the genera *Bacillus* and *Pseudomonas*, respectively. Some strains of *Actinomycetes* were also isolated. The dominance of bacilli in the samples can be explained by their ability to survive for a long time as spores. As to the fungi populations, the dominant strains identified were from the genera *Cladosporium* and *Penicillium*, although others strains were also isolated, namely *Aspergillus* sp., *Acremonium* sp., *Sporotrix* sp., *Trichoderma* sp.. However, several isolated mycelia did not present reproductive structures and were designated as sterile mycelia.

The microbial activity in all panels was assessed by enzymatic assays, namely, dehydrogenase (DHA). The method was optimized using INT (2-(4-iodophenyl)-3-(4 nitrophenyl)-5-phenyl tetrazolium chloride) with quantification of INT-formazan and applied for the dehydrogenase

determination in mortars and in *Penicillium sp.* cultures, which were selected in view of their predominant abundance in contaminated areas and to assess the dehydrogenase production.

The values of enzymatic activity in 3 mortar samples were  $1.023 \pm 0.049$ ,  $1.496 \pm 0.395$  and  $1.777 \pm 0.036$   $\mu\text{mol}/\text{min}/\text{g}$ . The enzymatic activity in *Penicillium sp.* cultures was evaluated for 5 days of culture. The growth profile of these cultures showed an exponential growth up to 72h. The enzyme activity of the cell fraction was top for the second day of culture, with a value of  $6.1\text{E-}01 \pm 1.64\text{E-}01$   $\mu\text{mol}/\text{min}/\text{L}$ . The results showed that there is a strong correlation between microbial activity and decay areas and that the enzyme dehydrogenase is a good biomarker for degradation assessment.



Fig.1. Details of the 16<sup>th</sup> century frescoes. The biological colonisations covering the frescoes are clearly visible.



*Bacillus sp.*



*Pseudomonas sp.*

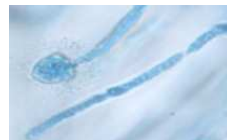
Fig.2. Some bacterial strains identified by optical microscopy

*Penicillium sp.*  
White mycelia wrinkled with greyish conidia



*Cladosporium sp.*  
Dark green, sulcate and wrinkled colonies

*Aspergillus sp.*  
Greyish green colonies



*Acremonium sp.*  
White and sulcate colonies.

*Sporotrix sp.*  
White and sulcate colonies



*Trichoderma sp.*  
White mycelium and grey conidia

Fig.3. Some fungi strains identified by optical microscopy

#### References

- [1] Bergey's Manual of Systematic Bacteriology, G.M. Garrity, D.R. Boone, R.W. Castenholz Eds., 2<sup>nd</sup> Ed., Springer, 2001, 721 pp.  
[2] Domsch, K.H., Gams, W. & Anderson, T.H. Compendium of Soil Fungi. Academic Press, London, 1980, 850 pp.

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