Microbiological Research **165** (2010) 21—32

Available online at www.sciencedirect.com

ScienceDirect

Microbiological Research

www.elsevier.de/micres

Heterotrophic microorganisms in deteriorated medieval wall paintings in southern Italian churches

O. Pepe[a,*], L. Sannino[b], S. Palomba[a], M. Anastasio[c], G. Blaiotta[a], F. Villani[a], G. Moschetti[d]

[a]*DSA Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, 80055 Portici, Italy*
[b]*SIB Società Italiana di Biotecnologie, 80055 Portici, Italy*
[c]*EURECO European Environmental Company, 81015 Piana di Monte Verna, Italy*
[d]*S.EN.FI.MI.ZO Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Università degli Studi di Palermo, 90128 Palermo, Italy*

Received 22 January 2008; received in revised form 18 March 2008; accepted 21 March 2008

KEYWORDS
Wall paintings;
Heterotrophic microorganisms;
Identification;
Deterioration

Summary

The Campania region in southern Italy is noted for its large number of churches that harbour invaluable frescoes, dated from the beginnings of the 4th up to the 13th century. The wall paintings represent an integral part of the monuments, and their deterioration constitutes a potentially significant loss for the world's cultural heritage. Heterotrophic microorganisms such as bacteria and mould can grow on the surface of paintings that contain a wide range of organic and inorganic constituents, and provide different ecological niches that are exploited by a large variety of microbial species. We isolated and identified the heterotrophic microorganisms found in the biodegraded medieval wall paintings of seven historical churches in Campania. The paintings showed different levels of microbial contamination. Microbiological analysis of different paintings gave an overview of the different heterotrophic microorganisms. Bacteria and moulds were isolated from 77% of the sampling points analysed, in which the most common type of alteration was discolouration often associated with detachment of the paint layer. Bacterial strains were identified by 16S rRNA partial sequence analysis. The *Bacillus* genus was isolated in all churches, even though the type of species was variable, whereas all actinomycetes strains, isolated in five of the seven churches analysed, could be referred to the *Streptomyces* genus. The similarity of the sequences analysed of the 42 *Bacillus* spp., 2 *Paenibacillus* spp. and reference strains of different species showed that these bacteria differentiated in 14 groups. The most frequently

*Corresponding author. Tel.: +39 81 2539410; fax: +39 81 2539407.
E-mail address: olipepe@unina.it (O. Pepe).

0944-5013/$ - see front matter
doi:10.1016/j.micres.2008.03.005

occurring taxa were most closely related to *Bacillus cereus/thurigiensis/anthracis* and *Bacillus pumilus* groups. Thirteen *Streptomyces* spp. were differentiated in seven groups on the basis of neighbor-joining analysis of 16S rRNA. Fungi belonging to the genera *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria* were also isolated from deteriorated wall paintings.

Introduction

Biological deterioration of works of art is assuming ever-greater importance especially in Italy with its disproportionate share (60%) of the world's cultural heritage, only 30% of which is properly conserved. In particular, the region of Campania in southern Italy has a wealth of ancient paintings that harbour invaluable frescoes dated from the beginning of the 4th up to the 13th century. Wall paintings support the growth of microorganisms commonly involved in biodeterioration, contributing to the destruction of paint and its backing; they are an integral part of the monuments, and their deterioration constitutes a loss affecting a significant part of the world's cultural heritage. The subaerial environment of the churches represents a semi-closed area in which the microbial community is enclosed in a mesocosm, partially isolated from the external environment and relatively stable in comparison to outdoor locations (Gorbushina et al., 2004). Heterotrophic microorganisms such as bacteria and mould grow on the surface of paintings that contain a wide range of organic and inorganic constituents and provide different ecological niches that are exploited by a large variety of microbial species. In particular, wall paintings contain pigments suspended in water or oil, often in the presence of a binder such as casein and milk applied on the damp lime plaster. Moreover, the spectrum of compounds is further increased by those that are added at later times during retouching and restoration (Ciferri, 1999). The activity of fungal and bacterial species is supported by many factors such as relative ambient humidity, temperature fluctuations, light, the nature of nutrients on the material, its moisture content, physical properties of the surface of the object, moisture adsorption-emission mechanisms in the support, pH, dust, oxygen and carbon dioxide concentration in the atmosphere, and the presence of microclimates that may induce condensation (Valentín, 2003). However, the environmental conditions (humidity, temperature, light and pH) will develop specific microbial flora on a fresco and could exacerbate the damage caused by air pollution, biological attack and natural aging (Ciferri, 1999). The first colonizers are chemolithotrophic bacteria that induce biological corrosion of the building material by the release of acids (Tomaselli, 2003; Karpovich-Tate and Rebrikova, 1991). The occurrence of chemoorganotrophic bacteria has also been investigated for their capacity to produce organic acids that solubilize the mineral components of the cultural heritage and affect the colour of the substrate surface (Urzí et al. 1991; Tiano 1998; Perito et al. 2000; Perito and Mastromei, 2003; Tomaselli 2003). They are commonly found on inorganic substrates containing traces of organic compounds which settle on the masonry surface (Saiz-Jimenez, 1995, 1997; Zanardini et al., 2000, 2002). Heterotrophic bacteria include a variety of genera such as *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Paenibacillus*, *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Staphylococcus*, *Nocardia*, *Mycobacterium* and *Sarcina*, which are the most frequent species isolated from wall paintings (Bassi et al., 1986; Saiz Jimenez, 1997; Ciferri, 1999). Recently, the use of a polyphasic approach for the detection and identification of the bacteria isolated from biodeteriorated frescoes, as well as the study of the microbial community, highlighted several new genera previously not detected with conventional methods, indicating the large biodiversity found in such inorganic substrates (Rölleke et al., 1998, 2000; Gutner et al., 2000; Daffonchio et al., 2000; Saiz-Jimenez and Laiz, 2000. Schabereiter-Gutner et al., 2001, 2002; Heyrman and Swings, 2003).

The degrading activity of fungi on materials has been widely investigated and proved, demonstrating that most isolated *taxa* are common soil inhabitants. (Jeffries, 1986; Sorlini et al., 1987; Bravery, 1988; Sampo and Luppi Mosca, 1989; Garg et al., 1995; Ciferri, 1999; Gorbushina et al., 2004).

Specific metabolic activities have been employed to develop new bioremediation methods based on the use of microbial cells and enzymatic activity to remove organic material (Ranalli et al., 2000, 2005; Beutel et al., 2002; Antonioli et al., 2005) or bioinduce calcite precipitation using specific bacteria for monumental stone reinforced (Castanier et al., 1999; Tiano et al. 1999; Fernandes 2006). Moreover, bioremediation of artworks is also based on the use of sulphate-reducing bacteria, which reduce