Biodeterioration of paintings is a field of multidisciplinary interest that draws from the arts, humanities and many branches of science. Medieval manuscripts comprise complex mixtures often toxic, mineral compositions (Cennini, 1390), containing metals such as arsenic, mercury, lead, iron, aluminium and cadmium (Ciferri, 1999), that may remain stable for long periods (Milanesi et al. 2006a). Recent restoration of the Chapel of the Holy Nail in Siena was a good opportunity to study deteriorated parts of a fresco by Lorenzo di Pietro, also known as ‘Il Vecchietta’ (Vasari, G., 1535). The composition of tiny fragments of five gold areas showing oxidation and blackening was analysed by scanning microscopy with x-ray dispersion (fig. 1 to 5 B). Only two samples (figs. 1 to 5 A) showed the indication and two incubated in solid medium (figs. 1 to 5 d) and then observed by scanning electron microscope. The compact surface of the original fragments (figs. 1 to 5 a), the absence of quiescent spores after hydration and culture, neutral pH and the absence of iron and copper enabled us to exclude proliferation of quiescent fungi and hyphae on the frescoed surface. We therefore analysed the microbial community for microbial diversity using 16S rRNA gene clone libraries. After total DNA extraction from the five fragments, only sequences from the part of the fresco illustrated in figure 3 were positive and two libraries were made: one from an original fragment and a second from a fresco fragment cultured in mineral medium without carbon source for quiescent bacteria to grow on endogenous carbon sources in the fragment. In the original untreated fragment two genera of Firmicutes were found: Bacillus unclassified bacterium X20 and Beijerinckia thermohalinae TIE1. This is in line with the literature which indicates that only 11-10% of bacteria present and quiescent in environmental fragments can be cultured in the laboratory. This result is also compatible with the genera of the Firmicutes, that are Gram-positive and produce microcrystals resistant to desiccation. Extraction of the cultured fragment confirmed the presence of about 95% sequences belonging to Streptococcus (27%) followed by non-culturable groups belonging to Burkholderiales incertae sedis (10%), pathogenic species of the class Neisseriaceae (4%) and Moraxella (2%). We also found Corynebacterium (1%) and Kocuria bacteria (2%) which tolerate sodium chloride but not acid pH (Milanesi et al. 2006b). Commensal bacterial such as Bacillus subtilis (4%) and Faecibacillus (1%) and a few bacteria typical of the microflora of heritage goods, such as Bacillus and Actinomyces. Interestingly, fragment 3 was similar to that associated with human in closed environments and hospitals (65%) as well as bacteria from a few days of human and animal origin. The absence of copper. When we surveyed microbial community diversity by 16S rRNA gene clone libraries, only fragments from one area were positive to DNA extractions. Two genera (Bacillus sp. and Beijerinckia sp.), both belonging to the Firmicutes, were found. Ninety-five clones of bacteria belonging to different taxa were found in the cultured fresco sample. The largest groups were Firmicutes (44%), most of which belonged to the genus Streptococcus. Microflora analysis showed 65% of clones known provenance; microflora resembled bacteria associated with humans in closed environments and hospitals, including agents of infection and disease (19%).

Results