



## Microbial monitoring in treated stone at the Royal Chapel of Granada

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Biom mineralization processes have been applied in situ to protect and consolidate decayed ornamental stone of the Royal Chapel in Granada (Spain). In few years, this conservation treatment has gained worth attention as environmentally friendly methodology for protection and consolidation of limestone because of the compatibilities shown between the new calcium carbonate cement and the original stone substrate. Moreover, the success of this approach may be related to the diversity of the microbiota inhabiting the stone and activated upon the biotreatment application and throughout the time. González-Muñoz et al. (2008) proposed a nutritional solution that activate among the bacteria inhabiting the stone those with carbonatogenic activity. In this study, a long-term (one, two and three years) monitoring of the microbiota present on the treated and untreated stones was done using a molecular strategy, including total DNA extraction, PCR amplification of 16S rRNA sequences, construction of clone libraries and fingerprinting by DGGE (Denaturing Gradient Gel Electrophoresis) analysis. Sequencing of the 16S rDNA revealed the dominant occurrence of members of Actinobacteria (44.20%), Gamma-proteobacteria (30.24%) and Chloroflexi (25.56%) after one year of the biotreatment. Whereas after two years, members of Cyanobacteria (22.10%) appeared and three years after, the microbiota consisted of only Actinobacteria and Cyanobacteria with approximately the same percentage in comparison with the untreated stones, dominated exclusively by Actinobacteria (100%). Fungal diversity followed the same dynamic as bacterial diversity being Ascomycota the predominant order before treatment. After one year, members of Basidiomycota and Viridiplantae appeared on the stone while two years after, the Viridiplantae dominated with a percentage of 84.77%. Finally, three years after the treatment, fungi population started to stabilize again and Ascomycota predominated next to 16.67% of Viridiplantae. The molecular strategy used here is proposed as an effective monitoring system to evaluate the impact of the application of bacterially induced calcium carbonate mineralization as restoration/conservation procedure for decayed ornamental stone. Moreover, these results confirm that the biotreatment, proposed by González Muñoz et al (2008), is friendly for the microbiota inhabiting the stone, which reaches stability after relatively few years.

References: González-Muñoz et al. (2008) Spanish Patent no. 2008/009771

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