Fluorescence *In Situ* Hybridization: a potentially useful technique for detection of microorganisms on mortars

Marina González, Ricardo Vieira, Patrícia Nunes, Tânia Rosado, Sérgio Martins, António Candeias, António Pereira and Ana Teresa Caldeira

ABSTRACT

This paper discusses the possibilities of applying Fluorescence In Situ Hybridization (FISH) to detect microorganisms on mortars, as this analytical technique has been used in different fields for the detection and identification of individual microbial cells in situ. FISH technique was applied for microbial detection on test and real mortars inoculated with fungal suspensions of S. cerevisae 396 and Nectria sp. A universal eukaryotic probe (EUK516) labelled with fluorescent dye (Cy3) was tested with different cell fixation procedures (4% (w / v) paraformaldehyde or 50% (v / v) ethanol in PBS). Positive results were obtained with FISH detection of Nectria *on testing/artificial as well as authentic/historical mortars, which confirms successful application of FISH technique to a new on mortars.*

1. Introduction

Microbial activity plays an important role in the deterioration of built heritage. Although the influence of microorganisms on deterioration processes is undisputable, the role of individual microbial species that form the communities is not yet fully understood. The development of new microbial detection and identification techniques is crucial for furthering our knowledge of microbial influence on heritage deterioration and designing appropriate preservation strategies.

Detection and identification of microbial communities present on artwork can be achieved using various complementary methods, with new approaches being continuously developed. The traditional culture-based techniques are time-consuming and are limited by the microorganisms' ability to grow under standard laboratory cultivation conditions [1, 2]. To overcome this drawback, culture–independent techniques based on molecular approaches, that are more sensitive and need smaller quantities of sample than those previously mentioned, have been applied. The use of molecular techniques based on expensive Polymerase Chain Reaction (PCR) present an important limitation, i.e. the impossibility of studying the microorganisms *in situ* [1, 2]. Nevertheless, a "non-PCR"-based technique is available that combines the precision of molecular techniques with providing information on the number and spatial distribution of microorganisms: Fluorescence *In Situ* Hybridization (FISH) [3]. As well as being inexpensive and informative, this analytical technique is also very powerful, rapid and straightforward [4]. Surprisingly, only a few studies in the field of cultural heritage