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Analysing bacteria and fungi colonising Cultural Heritage by Fluorescence *In Situ* Hybridisation

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Deterioration of Cultural Heritage (CH) materials due to microbial proliferation is an inherent problem of artworks existence. Our group is focused on exploiting the potential of Fluorescence *In Situ* Hybridisation targeting ribonucleic acid molecules (RNA FISH) for analysing the microorganisms involved in this process. RNA FISH technique allows simultaneous detection of various microbial populations of interest by using probes labelled with different fluorescent dyes [1]. It should be particularly interesting to adapt the FISH protocols for assessing simultaneously the presence of fungal and bacterial colonisation of CH. However, it is well known that the rRNA content and the properties of the cellular walls vary between microorganisms and strongly influence the RNA FISH results [1]. In fact, it is usual to apply specific FISH protocols for analysing each type of microorganism.

Thus, the main outcome of this work was the development and optimisation of a common and rapid RNA FISH protocol for analysing fungi (yeast and filamentous fungi) and bacteria. The first goal of this work was to investigate the influence of various factors of the FISH protocol (probe and cell concentration, fixation conditions) and properties of the yeast and bacteria cells (physiological state, enzymatic activity and RNA content) on the RNA FISH results, qualitatively (by epifluorescence microscopy) and quantitatively (by flow cytometry). The FISH protocol developed by us for analysing filamentous fungi in suspension were used as a first approach to analyse the cells harvested from pure liquid cultures of yeast (*S. cerevisiae* and *Rhodotorula* sp) and bacteria (*Bacillus* sp and *E. coli*) [2]. The physiological state of the cells, their RNA content and enzymatic activity, as expected, show strong influence on the results. However, good FISH signals have been obtained, independently of the variation of these cell properties, by fixing the cells in ethanol/PBS solutions (50, 80 and 100% v/v) for 3, 15 and 60 min. The concentration of the cells and of the FISH probes, EUK516-Cy3 and EUB338-Cy3, reveal to be crucial factors for yeast and bacteria detection. Thus, they need to be controlled for ensuring the detection of these microorganisms.

Since: I) yeast and bacteria have been successfully detected by applying the FISH protocol independently of the fixation conditions (ethanol/PBS solutions 50, 80 or 100% v/v for 3, 15 and 60 min); and II) these conditions have also been previously applied by us for analysing filamentous fungi isolated from the Portuguese CH obtaining good results, this work have result in the development of a rapid FISH protocol for analysing fungi as well as bacteria in suspension, reducing the fixation time to 3 min. This will be the basis for using RNA FISH for simultaneous analysis of these microorganisms in CH materials.

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[1] A. Moter. U. B Göbel. Journal of Microbiological Methods, 41, 2000, 85-112.

[2] M. González, R. Vieira, P. Nunes. T. Rosado, S. Martins, A. Candeias, A, Pereira, A. T. Caldeira, In e-conservation Journal, (2014) pp 44-52.