Detecting cells with low RNA content colonizing artworks non-invasively: RNA-FISH

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There is a need to enhance the methods for signalizing the microorganisms associated to biodeterioration of Cultural Heritage (CH) materials. An RNA-FISH in Suspension protocol was previously proposed by us as an alternative for *ex situ* analysis of the microbial colonizers from microsamples [1]. However, since: i) the low RNA content of the target cells has been previously reported as a possible limitation for RNA-FISH application in environmental and CH samples; and ii) the use of non-invasive sampling methods is preferred in CH field; the aim of this work was to investigate the capacity of this RNA-FISH protocol to detect cells with low RNA content and the possibility of adapting it for application in samples collected by non-invasive techniques.

Universal probes for targeting Eukaryotes (EUK516) and Bacteria (EUB338) labeled with Cy3 and 6-FAM dyes were used for all the assays. Cells with low RNA content (checked through RNA extraction and spectroscopic quantification) of bacteria and yeast strains isolated from biodegraded Cultural Heritage objects were used to simulate those colonizing artworks. For evaluating the possibility of the protocol to become a non-invasive approach, wood and stone slabs artificially inoculated with biodeteriogenic yeast and bacteria cells were prepared and a low invasive sampling was performed by swabbing or by an impression method with filter paper, nitrocellulose and two types of membranes. The cells were recovered from the samples and the RNA-FISH protocol was applied in the resulting suspensions. Regarding the detection of cells with low RNA content, the results showed that, for all the microorganisms tested, using the specific probes for the target microorganisms labeled with the Cy3 dye satisfactory signals were observed. However, low intense or undetectable signals were detected with 6-FAM labeled probes. This revealed the potential of the RNA-FISH In suspension protocol to detect bacteria and yeast cells with extremely low RNA content and evidenced the need of using dyes with high quantum yields for avoiding false negatives.

On the other hand, when the sampling was performed by swabbing or by the impression method and this RNA-FISH approach was applied: i) the cell recoveries obtained for all the methods before FISH application were acceptable; and ii) good FISH signals were detected without background interference.

Thus, whereas more tests are required, the non-invasive RNA-FISH methodologies presented in this work seem to be good alternatives for analyzing the potential biodeteriogenic microorganisms thriving in CH objects overcoming the drawback of background fluorescence.

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[1] Vieira, R. et al. *Conservar Património*, 2016, 23: 71.