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A novel Rhodotorula sp. RNA-FISH probe for the identification of this artwork's biodeterioration agent

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Microorganisms with biodeteriorative potential can produce serious damage to materials of tangible cultural heritage (e.g. canvas, paper and stone). Some bacteria and yeast, as result of their metabolic activity, can produce carotenoid compounds leading to colour alteration of these materials. The yeast *Rhodotorula sp.* is a pink-red carotenoids producer whose colonisation has been reported to be associated to the apparition of pink stains on the walls of the Cathedral of one of the most emblematic monuments of Évora, Portugal [1]. To distinguish *Rhodotorula sp.* from other microorganisms that produce pink-red carotenoids proper identification methods must be applied such as RNA-Fluorescence In Situ Hybridisation (FISH) that allows identification of individual cells in microbial communities. This technique is based on hybridisation of fluorescently-labelled oligonucleotide probes targeting to specific regions of the ribosomal RNA [2]. Thus, the aim of this study was to design *in silico* a novel RNA-FISH probe to *Rhodotorula sp.*, that facilitate a precise identification of this yeast, and to evaluate its specificity and experimental performance. The probe designed was a novel 26S RNA probe whose experimental evaluation was done by constructing the fluorescence-signal-response/formamide concentration curve for the target and a non-target yeast (*Cryptococcus adeliensis*). A previously described FISH procedure was applied [3] and the fluorescence intensities were measured by flow cytometry. *In silico* and experimental results revealed that the *Rhodotorula sp.* probe is genus-specific. Hence, this study will contribute to an accurate identification of microorganisms involved in the biodeterioration process, advantageous for the selection of proper remediation strategies.

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