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Image: Marble head representing Julius Cesar circa 20 B.C. – 10 A.C.
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Experimental specificity and performance of two rRNA FISH probes designed to detect and identify Cladosporium sp.

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Fungi are common colonizers of artworks involved in their aesthetic and structural changes. Thus, detection and identification of filamentous fungi is of special interest for the safeguarding of Cultural Heritage objects [1,2]. The viable microorganisms thriving in them have been successfully detected and identified by Fluorescence In Situ Hybridization (FISH) [3]. Cladosporium sp. are among the most common microbial colonizers of art objects [4]. Thus, the aim of this study was to investigate the experimental specificity and performance of two rRNA FISH probes designed in silico by us to detect and identify fungi belonging to this genus. (CLAD2.1 and CLAD5). Suspensions of spores/conidia of the target (Cladosporium sp.) and non-target (Penicillium sp.) molds isolates were used. The specificity of the probes was evaluated varying the formamide concentration in the hybridization and washing buffers. Their performances were assessed by epifluorescence microscopy and flow cytometry, respectively. Five RNA-FISH assays were performed in each condition for each isolate (a blank, the negative and positive controls and the tests with the Cy3-labelled designed probes: CLAD2.1-Cy3 and CLAD5-Cy3 tests) following a protocol previously stablished by our group [3]. The experimental analysis revealed that, independently of the concentration of formamide used: i) fluorescent non target conidia/spores were only observed for the positive controls; and ii) Cladosporium spores/conidia showed fluorescence for the positive controls and CLAD5-Cy3 tests. This indicates that CLAD2.1 Cy3 fails to detect/identify Cladosporium sp. cells. However, CLAD5 Cy3 is a good alternative to do it, showing good performance and specificity to Cladosporium sp. in a wide range of formamide concentrations. Acknowledgements: This work was co-financed by ALT20-03-0246-FEDER-000004-ALENTEJO 2020 project and by FCT through PTDC/BBB-IMG/0046/2014 project and SFRH/BPD/100754/2014 grant. Bibliography: 1. Sterflinger, K., Fungal Biology Reviews, 2010. 24(1): p. 47-55. 2. Rosado, T., et al., International Biodeterioration & Biodegradation, 2013. 85: p. 1 7. 3. González-Pérez, M., et al., Applied Physics A, 2017. 123(2). 4. Rojas, T.I., et al., Grana, 2012. 51(1): p. 44-51.