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BOOK OF ABSTRACTS

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P-123 - FLUOROPHORE´S ROLE ON THE RELIABILITY OF MICROORGANISM DETECTION BY FLUORESCENCE IN SITU HYBRIDISATION (FISH)

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Abstract
FISH has been applied in many ecological and phylogenetic studies becoming the method of choice for the direct detection and identification of microorganisms in their natural environments [1]. For reliable results, it is crucial to minimise or avoid background fluorescence and cellular autofluorescence but also to maximise the specific FISH signals obtained. Red-emitting dyes have been usually used for eliminating the problems of non-specific fluorescence interference. Therefore, in this work we evaluated the role of three of these fluorophores (Alexa Fluor 647 (AF647), ATTO 647N and Cy5) on the reliability of the RNA-FISH results obtained both with a universal and a specie-specific probe.

Materials and Methods
RNA-FISH assays were performed using two strains of *Dekkera bruxellensis* (CBS 2797 and ISA 2101) following the protocol previously described by us [2]. Four assays were performed for each strain with the addition of different probes (1)-non-probe; (2) EUB338; (3) EUK516; (4) specific-RNA-FISH probe to *D. bruxellensis* (26S D. brux.5.1) previously described in the literature [3]. The probes used were: 5'-EUB338-, 5'-EUK516-, 5'-26S D. brux.5.1 labelled with AF647 and ATTO 647N as well as 5´-EUB338-Cy5 and 5´-EUK516-Cy5. Their performances were evaluated in terms of detectability and percentage/fluorescence intensity of the target cells, by epifluorescence microscopy and flow cytometry, respectively.

Results & Conclusions
As expected, no fluorescent signals were obtained without addition of probe and with EUB 338 labelled with the three fluorophores selected. The universal probe, EUK516, labelled with each of the fluorophores selected allowed the reliable detection of the yeast cells. Microscopic observations revealed that ATTO 647N-labelled probes conferred higher fluorescence stability to the hybridised cells than the other fluorophores tested (AF647 and Cy5). Likewise, by flow cytometry the best results were detected with EUK516 probe labelled with ATTO 647N. Nevertheless, cells stained with AF647 showed the lowest fluorescence intensities. Yeast cells stained with EUK 516 usually shows intense FISH signals. However, specie-specific probes can give weaker fluorescent intensities that can hinder the detection and identification of the target cells. Therefore, the fluorophores influence on the accurate detection and identification of the target cells was investigated using a specific RNA-FISH probe to *D. bruxellensis* (26S D. brux.5.1). Only, ATTO 647N and AF647 were selected as they showed the highest and lowest fluorescence with EUK 516 probe. The signals obtained by flow cytometry, even with low intensity, allowed to identify the cells hybridised with 5'-26S D. brux.5.1-AF647 as *D. bruxellensis*. However, no fluorescence was detected by epifluorescence microscopy. This indicates that this probe labelled with AF647 allowed the specific detection of *D. bruxellensis* by flow cytometry but not by epifluorescence microscopy. Conversely, intense fluorescent cells were detected when hybridised with the same RNA-FISH probe (5'-26S D. brux.5.1) but labelled with ATTO 647N both by epifluorescence microscopy and flow cytometry. Thus, this study highlighted that the selection of a fluorophore with high photostability and quantum yield, such as ATTO 647N, can improve FISH performance and contribute to avoid inaccurate identification of microorganisms by RNA-FISH technique independently of the method used for analysis.

References & Acknowledgments
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