Detection for yeast and bacteria in wood slabs by RNA-FISH

R. Vieira[1,2], A. Candeias[1,2], M. González-Pérez[1], A. T. Caldeira[1,2]

[1] HERCULES Laboratory, Évora University, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal

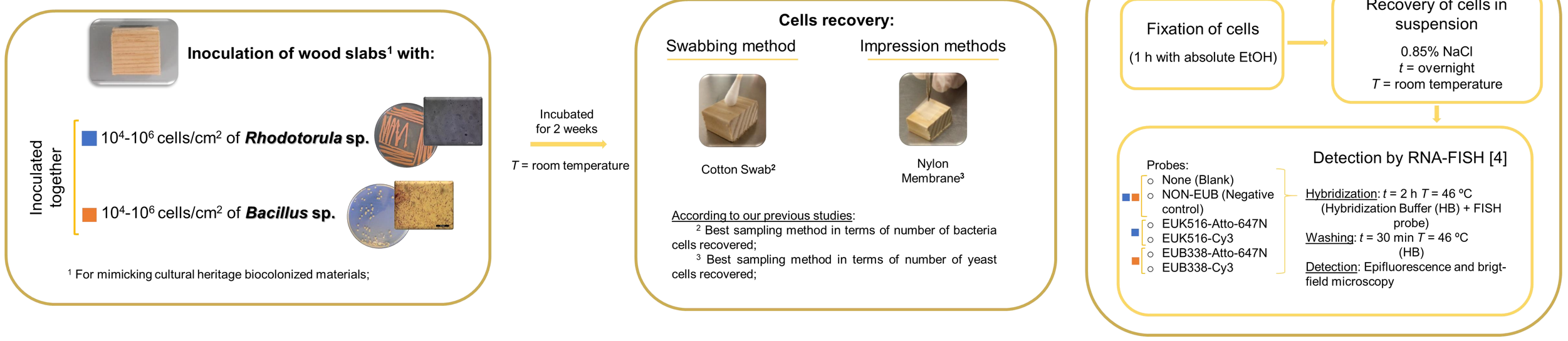
[2] Chemistry Department, School of Sciences and Technology, Évora University, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

1. INTRODUCTION

The deterioration of cultural heritage objects and assets (mural paintings, statues, and many other art objects made of wood, stone, paper, ceramic, glass, *inter alia*) can be caused by microorganisms [1]. One of the most important steps for applying adequate conservation and protection measures is early identification and monitorization of microbial colonization. The conventional culture-based methods used so far have become insufficient to detect/identify the biodeteriogenic agents. Thus, molecular techniques have started to attract considerable interest [2,3].

Our group is focused on detecting and identifying microorganisms that cause biodeterioration on artworks using the RNA-FISH molecular technique [4]. It is a simple, rapid and promising molecular technique enabling detection, visualization and identification of the viable microorganisms of interest [5,6]. As any other technique, RNA-FISH has its own Limit Of Detection (LOD), minimum detectable concentration of cells. For ensuring the reliability of RNA-FISH analyses, determination of the associated LODs is imperative. Thus, the aim of this work was to determine the LOD for yeast and bacteria in wood slabs by RNA-FISH. Universal probes for targeting eukaryotes (EUK516) and bacteria (EUB338) labeled with Cy3 or Atto-647N dyes were used.

2. EXPERIMENTAL PROCEDURE



3. RESULTS AND DISCUSSION

- In previous studies perfomed by our group it was found that, from the methods tested, the swabbing method with cotton swab is the method that allows to recover a higher number of bacteria cells, whereas the impression method with nylon membrane showed the best results in terms of recovery of yeast cells. Therefore, using these sampling methods, the LODs of the RNA-FISH technique for *Bacillus* sp. and *Rhodotorula* sp. detection/identification was determined.

Table 1. Epifluorescence and bright-field microscopy results for the cells stained by RNA-FISH with probes labeled with Cy3 and Atto-647N after their recovery, by swabbing or impression methods (for bacteria and yeast cells, respectively), from wood slabs inoculated with different concentrations of cells.

Number of cells inoculated per cm²	*Rhodotorula* sp.		*Bacillus* sp.	
	EUK516-Cy3	EUK516-Atto-647N	EUB338-Cy3	EUB338-Atto-647N
10^6	‡ ‡ ‡	‡ ‡ +	‡ + +	+ + +
10^5	+ + +	‡ ‡ +	‡ ‡ +	+ + +
10^4	- - -	- - -	- - -	+ + +

The signs indicate that the cells of *Rhodotorula* sp. and *Bacillus* sp. were detected with fluorescence (‡), only detected in bright–field without fluorescence (+) or not detected (-). The assays were done in triplicate.

- The microscopical observations with adequate filter sets revealed that the LODs for yeast are 10^6 cells/cm² with EUK516-Cy3 and 10^5 cells/cm² with EUK516-Atto-647N. The LOD for bacteria was found to be 10^5 cells/cm² using EUB338-Cy3. However, the LOD with EUB338-Atto-647N could not be determined but is is higher than 10^6 cells/cm².

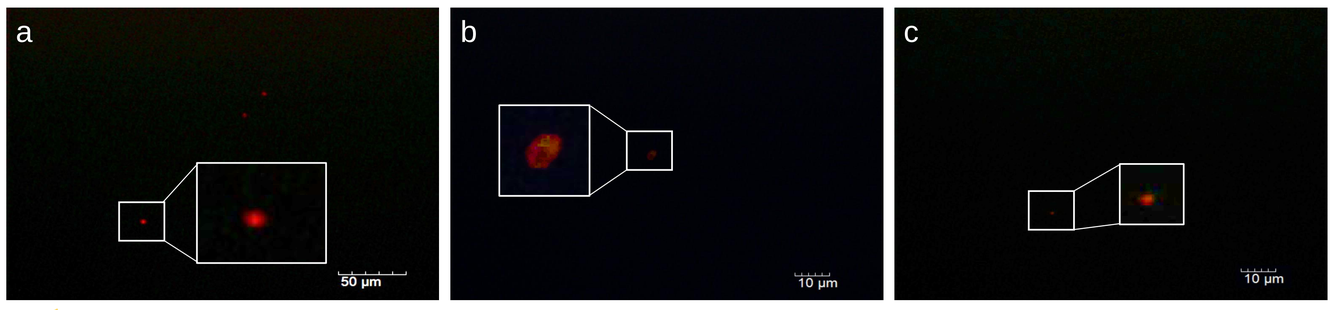


Figure 1. RNA-FISH signals obtained after 2 weeks for samples inoculated with 10^6 cells/cm² of Rhodotorula sp. and Bacillus sp. using EUK516-Cy3 (a), EUK516-Atto-647 (b) and EUB338-Cy3 (c). The probes labeled with Cy3 and Atto-647N were analyzed by the corresponding filter sets, TRITC and Cy5, respectively.

- With 10^6 inoculated cells per cm², good fluorescence signals were observed for the yeast with the dyes Cy3 and Atto-647N and good fluorescence signals were also observed for the bacteria with the dye Cy3. With the dye Atto-647N it was just possible to detect bacteria cells by bright–field microscopy;
- Bacterial and yeast cells did not show fluorescence on blank (none probe) and negative control (NON-EUB).

4. CONCLUSIONS

- LOD for yeast detection in wood by RNA-FISH: 10^6 cells/cm² for EUK516-Cy3 and 10^5 cells/cm² for EUK516-Atto-647N (2 weeks after inoculation);
- LOD for bacteria detection in wood by RNA-FISH: 10^5 cells/cm² for EUB338-Cy3 and higher than 10^6 cells/cm² for EUB338-Atto-647N (2 weeks after inoculation);
- The best dye to detect yeast cells with the RNA-FISH technique in combination with the nylon membrane is Atto-647N and to detect bacteria cells recovered by swabbing is Cy3.

ACKNOWLEDGMENTS

This work was co-financed by ALT20-03-0246-FEDER-000004-ALENTEJO 2020 project and by FCT through the project PTDC/BBB-IMG/0046/2014 and grants SFRH/BD/118028/2016 and SFRH/BPD/100754/2014.

5. REFERENCES

[1] Dakal, T. . *et al*. *Rev Environ Sci Bio/Technology*. 11 (2012):71-104.

[2]Lech, T. *et al*. IJCS. 6 (2016):137-144.

[3]Gonzalez, JM. *et al*. *J Sep Sci*. 27 (2004):174-180.

[4]Vieira, R. *et al*. *Conservar Património* 23 (2016): 71–77.

[5]Aoi, Y. *J Biosci Bioeng*. 94 (2002):552-556.

[6]Bottar,i B. *et al. Appl Microbiol Biotechnol*. 73 (2006):485-494.