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BOOK OF ABSTRACTS

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P-082 - SPECIES-SPECIFIC RNA-FISH PROBES FOR YEAST IDENTIFICATION: EVALUATION OF THEIR SPECIFICITY AND PERFORMANCE

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Background

RNA-FISH is a powerful molecular technique that allows identification of individual microbial cells. The application of a unique protocol for microbial identification from the kingdom down to the species level simplifies the analytical procedure. Recently, our research group has developed an efficient RNA-FISH protocol for simultaneous analysis of fungi and bacteria using kingdom-specific probes. Thus, the aim of this work was to investigate the possibility of applying this protocol for specific detection of various yeast species using previously published species-specific probes. Their specificity and performance were analysed both *in silico* and experimentally.

Method

Suspensions of target (*Cryptococcus adeliensis*, *Dekkera bruxellensis and Zygosacharomyces bailii)* and non-target yeast isolates (*Lachancea thermotolerants, Pichia kudriavzevii, Rhodotorula sp., R. mucilaginosa, Saccharomyces cerevisiae, Hanseniaspora guillermondii* and *Torulaspora delbruekii*) were used. The autofluorescence of the cells was evaluated by epifluorescence microscopy using Cy3, FITC and Cy5 filter sets. The specificity and hybridization efficiency of the probes were evaluated *in silico* using NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and mathFISH (http://mathfish.cee.wisc.edu/). Seven different RNA-FISH assays were performed for each isolate following the protocol previously described by us [1]: (1) blank without probe addition; (2) negative control with EUB338; (3) positive control with EUK516; (4-7) test assays with *Z. bailii*, *D. bruxellensis*, *Brettanomyce*s and *C. adeliensis* previously published probes [2-4]. All probes were labelled with Alexa Fluor 647 (AF647) at the 5′-end.

Results & Conclusions

The microscopic observation of the target cells revealed that for avoiding false positives, associated to autofluorescence, a red-emitting dye could be used. Thus AF647-labelled RNA-FISH probes were applied in this study. The *in silico* analysis revealed that the probes tested possess not only a high specificity but also a high theoretical hybridization efficiency. This was confirmed by the experimental approach. The analysis of RNA-FISH treated yeast cells also showed that the FISH protocol, previously used for identification of fungi and bacteria, is suitable for specific detection of several yeast species. Therefore, the possibility of simultaneous identification of these microorganisms from the kingdom to the species level with a single protocol and with a good FISH performance looks promisor.

References & Acknowledgments

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