P Branco et al., J Microb Biochem Technol 2017, 9:6(Suppl)
DOI: 10.4172/1948-5948-C1-035

A novel *Dekkera bruxellensis* RNA-FISH probe: Design and evaluation

P Branco, A Candeias, A T Caldeira and **M Gonzalez Perez**
Evora University, Portugal

RNA-fluorescence *in situ* hybridization (FISH) technique is based on the hybridization of fluorescently-labelled oligonucleotide probes targeting to specific regions of the ribosomal RNA. Its use has exponentially increased in the last decades for microbial identification. However, one of the main limitations of this technique is the reduced number of specific RNA-FISH probes available. One of the key issues is the design of probes with the desired level of specificity and high hybridization efficiency. Specificity of probe binding to the target site depends on the stringency conditions, commonly adjust using formamide. Thus, *in silico* and empirical formamide denaturation curves are commonly used searching for the optimal conditions for ensuring specificity. Therefore, this work was focused on the design of an RNA-FISH probe target to a yeast specie (*Dekkera bruxellensis*) and on the evaluation of its suitability *in silico* and experimentally. The probe was designed *in silico* by using DECIPHER program. It was analyzed with mathFISH program and blast nucleotide to calculate hybridization efficiency and specificity/coverage, respectively. Experimental evaluation was done by constructing the fluorescence-signal response/formamide concentration curve for the target and a nontarget yeast (*Candida krusei*). A previously described FISH procedure was applied, and fluorescence intensity was measured by flow cytometry. Excellent performance of the probe was observed with high maximal theoretical *in silico* hybridization efficiency (99.89%) and specificity. The experimental evaluation revealed that the probe has high specificity with 5% of formamide showing its maximal fluorescence signal response for the target and none for the non-target yeast.

Biography

P Branco has completed her Master's in Molecular Biology from the Institute of Health Science Egas Moniz, Monte da Caparica, Portugal and PhD in Food Engineering from the Higher Institute of Agronomy, University of Lisbon, Portugal. She has published seven papers in peer reviewed journals. Since May 2017, she is working as Researcher in the Hercules Laboratory at the Évora University where she is focused on the development of new RNA-FISH probes for identification and detection of microorganisms involved in biodeterioration of cultural heritage in MICROTECH-ART project funded by FCT (Fundação para a Ciencia e a Tecnologia).

~~aurelian.rotaru@usm.ro~~

Notes: