PT029 Optimizing locked nucleic acid/2'-o-methyl-rna fluorescence in situ hybridization (lna/20ome-fish) procedures for bacterial detection

Andreia Azevedo[1;2;3;4], Ricardo Fernandes[5], Nuno Azevedo[5], Carina Almeida[6]

[1]*Centre of Biological Engineering, University of Minho, Braga, Portugal*
[2]*FEUP - Faculdade de Engenharia da Universidade do Porto, Laboratory for Process Engineering, Environment, Biotechnology and Energy, Porto, Portugal*
[3]*IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal*
[4]*i3S - Instituto de Investigação e Inovação da Universidade do Porto, Porto, Portugal*
[5]*FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, Portugal*
[6]*INIAV - Polo de Vairão, Vairão, Portugal*

Background: Despite the successful application of locked nucleic acid/2'-O-methyl-RNA fluorescence *in situ* hybridization (LNA/2'OMe-FISH) procedures for bacteria detection, there is a lack of knowledge on the properties that affect hybridization. Such information is crucial to find the more suitable hybridization conditions for bacteria detection either in an individual or in a multiplex assay.

Objectives: This work aimed to evaluate the effect of three essential factors on the LNA/2'OMe hybridization step - hybridization temperature, [NaCl] and type/concentration of denaturant (formamide, ethylene carbonate and urea).

Methods: The optimization was performed for 3 Gram-negative bacteria (*Escherichia coli* CECT 515, *Citrobacter freundii* SGSC 5345 and *Pseudomonas aeruginosa* PAO1) and 2 Gram-positive bacteria (*Enterococcus faecalis* CECT 184 and *Staphylococcus epidermidis* RP61A), using an Eubacteria LNA/2'OMe probe (5'mTlGlCmClTmCmClCmGmTlAmGmGlA3'; "l" - LNA; "m" – 2'OMe). The signal quantification was evaluated by flow cytometry and Response Surface Methodology was used to model the interaction between the 3 parameters.

Results: It was observed that a high NaCl concentration is beneficial (2M-5M), regardless of the denaturant used. Urea, formamide and ethylene carbonate are suitable denaturants for LNA/2'OMe-FISH applications; but urea provides higher fluorescence intensities among the different bacteria. The results indicate that a hybridization solution with 2M of urea and 4M of NaCl would be a proper starting point for multiplex LNA/2'OMe-FISH procedures. Furthermore, a hybridization temperature around 62°C, for 14bp probes with LNA monomers at every third position of 2'OMe might be use in initial optimizations.