

Efficiency of enzymes and benzalkonium chloride treatments against *Listeria monocytogenes* dual-species biofilms determined by fluorescence microscopy and image analysis

RODRÍGUEZ LÓPEZ Pedro, NIMO FERNÁNDEZ Vanessa, CARRERA IGLESIAS Ana Belén,
BLANCO MARTÍN Teresa, LÓPEZ CABO Marta

*Seafood Microbiology and Technology Group, Marine Research Institute - Spanish National Research Council (CSIC),
C/ Eduardo Cabello, 6, 36208 Vigo (Spain)*

In the present study, three different mixed-species *Listeria monocytogenes*-carrying biofilms previously isolated from surfaces of seafood, meat and dairies industrial environments were selected in order to test the efficiency of cleaning and disinfection procedures based on the combination of an enzymatic solution, followed by a treatment with benzalkonium chloride (BAC).

Biofilm samples were cultured onto AISI-316 type stainless steel 1 cm² coupons in a batch system at 25° C. The effect of sequential exposure to different concentrations of Pronase - BAC, Cellulase - BAC or DNaseI - BAC solutions was then assessed in mature biofilms after 168 hours using an orthogonal first order experimental design. Samples were stained using FilmTracer™ LIVE/DEAD® Biofilm viability kit (Invitrogen) and the efficiency of each treatment was then determined by epifluorescence microscopy and subsequent image analysis of 25-field mosaics representing a total surface of 1.92 mm², so as to quantify the total area occupied by living and damaged cells attached to the surface.

Significant empirical equations obtained reveals that synergistic effects between Pronase - BAC on biofilms coming from fish and dairy industries obtaining almost a total cell detachment in the latter case. No significant effect was observed among the Cellulase - BAC procedures in the experimental ambit assayed, being in some cases even counter-productive. A lower effect of the combination DNaseI - BAC against biofilms coming from fish and dairy industries compared with the other two combinations enzyme - benzalkonium chloride was also demonstrated even with no effect against the consortium isolated from meat industry.

These results show how the usage of customised treatments combining commercially available enzymes with classical chemically-based disinfection procedures may represent an attractive and efficient alternative against bacterial biofilms present in the food industry.