Title: Influence of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) on dispersal *of Listeria monocytogenes* in biofilms.

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Surface-associated communities of micro-organisms, known as biofilms, must release and disperse cells into the environment to colonize new sites. Three modes of biofilm dispersal are known: erosion (continuous release of single cells or small clusters of cells), sloughing (sudden detachment of large portions of the biofilm), and seeding (or central hollowing - which refers to the rapid release of large number of single cells or small clusters of cells, forming cavities inside the biofilm). Processes of erosion and sloughing can be active or passive but, seeding dispersal is always an active event. The elucidation of molecular mechanisms of biofilm dispersal is key to plan strategies to get rid of bacterial contamination in food processing premises. At molecular level, it has been shown that oxygen and nitrogen reactive species (ROI and RNI, respectively) may trigger cell detachment from biofilms in many bacterial species, but the roles of ROI and RNI are not completely clear. In this study, the presence of oxidative and nitrosative stress markers was evaluated in 4 and 8-days old biofilms formed by the foodborne pathogen *Listeria monocytogenes*. The effects of one nitric oxide donor (sodium nitroprusside) and two inhibitors [N-nitro-L-arginine methyl ester and 2-(4-carboxyphenyl)-4,4,5,5-tetrametilimidazoline-1-oxy-3-oxide] were also tested. Biofilms of *L. monocytogenes* grown on stainless steel and glass surfaces were monitored by culture and by microscopic techniques (fluorescence and confocal laser scanning microscopy). Nitrite - a nitric oxide precursor - was quantified in biofilms with Griess reagent. Reverse Transcriptase Real-Time PCR was used to evaluate the expression of the regulatory gene *prfA* and others, related to ROI and RNI (*lmo 0990*, *lmo 0807* and *lmo1485*). The results indicated NO, peroxynitrite, H_2O_2 and superoxide radicals were present in biofilms of *L. monocytogenes*. Donor or inhibitors of NO did not inhibit listerial growth and, the expression of the gene *lmo0990* was the only one modified (downregulated) by the chemicals tested. Overall, the results indicated ROI and RNI did not act as dispersive agents of *L. monocytogenes* in biofilms.

Keywords: *Listeria monocytogenes*, biofilms, dispersal, nitric oxide

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