The adhesion control of *Listeria monocytogenes* on food-processing surfaces by silver ion implantation

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Bacterial adhesion to a solid surface is a crucial step in the biofilm process. *Listeria monocytogenes* can adhere to food-processing surfaces, survive and grow over a wide range of environmental conditions such as refrigeration temperatures and consequently cause severe disease. Several strategies have been developed with the aim to decrease the adherence of bacteria to surfaces, namely the silver ion implantation on surfaces. Thus, the aim of this work was to determine the *Listeria monocytogenes* CECT 4031 T adhesion ability onto four types of AISI 304 and AISI 430 surfaces usually used in food industry, restaurant, and kitchens, and simultaneously to evaluate the influence of the thermodynamics aspects on bacteria adhesion on these different surfaces.

Coupons (1 cm^{-2}) were cut from a 1 mm layer of AISI 304 and AISI 430 surfaces (N° 2B, 4 , 6 and 8). The silver ions (Ag^{+1}) were implanted at 200 keV, 1.0 $\mu A.cm^{-2}$ and a dose of 2.0×10^{16} $ions.cm^{-2}$. All coupons were cleaned by immersion in 0.2% solution of a commercial detergent for 5 min, followed by immersion in ethanol for 15 min. The coupons were twice rising with ultrapure water and dried at 60 °C. Each strain was subcultured in trypticase soy broth (TSB) at 37 ºC in an orbital shaker (120 rpm), overnight. The cells were then harvested by centrifugation at 9000 rpm for 5 min and washed twice with phosphate buffered saline (PBS 0.1M pH 7). The pellets were resuspended in PBS to an inoculum level of 10^9 $CFU.ml^{-1}$, determined by optical density. Adhesion assays were performed in sterile 24-well microtiter plates and each well was filled with 970 µl of TSB supplemented with 0.6% (w/v) of yeast extract, 30 µl of cell suspension and the respective coupon. The plates were incubated at 4 ºC for 2 h, with constant agitation at 120 rpm. After incubation, the coupons were washed once with 1.0 ml of minimal medium (MM) to remove non-adherent cells and replaced to a new well and the adhered cells were removed by scrapping on 1.0 ml of MM carefully. The number of viable cells was quantified by colony forming units (CFUs) on trypticase soy agar (TSA). The materials and *Listeria* cells hydrophobicity properties were evaluated through contact angle measurements and using the approach of van Oss and coworkers.

The results showed that the strain used was able to adhere to all materials. It was not found significant differences ($p > 0.05$) between the means of the *L. monocytogenes* adhered cells on the twelve surfaces studied. However, the highest mean value of adherence cells occurred to AISI 304 N° 4 (4.78 ± 0.32 log $CFU.cm^{-2}$) without silver ion implantation (wi) surface. Moreover, it was possible to observe that AISI 430 N° 8 with silver ion implantation (i) (4.29 ± 0.37 log $CFU.cm^{-2}$) and AISI 430 N° 4 wi (3.60 ± 0.31 log $CFU.cm^{-2}$) surfaces presentes the lowest means ($p < 0.05$). Concerning hydrophobicity, silver ion implantation increase the hydrophilicity of the surfaces, except in case of AISI 304 N° 6 ($p.> 0.05$). Furthermore, the results showed that *L. monocytogenes* cells are hydrophilic. Moreover, no correlation was observed between the number of adhered cells and substrate surface hydrophobicity, despite of the highest number of bacteria cells adhered mainly occured on surfaces with highest water contact angle value.

The contact time between microorganism and silver implantated stainless steel surfaces seems not to be enough to confer antimicrobial activity. So, we consider that more studies are necessary to evaluate the effective effect of silver as antimicrobial agent to control the adhesion of *L. monocytogenes* cells and biofilms formation. As future work, we will study the effect of silver ion as antimicrobial different time periods.

Keywords: Silver ion implantation; Food-processing surface; Control adhesion; *Listeria monocytogenes*.