TITLE: The effect of light and storage temperature on attachment and internalization of *Salmonella* Typhimurium in lettuce (*Lactuca sativa* var *crispa*) leaves

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ABSTRACT:

Salmonella Typhimurium is able to attach to the surface of plants and progress to the internal structures, hampering the efficacy of the cleaning and disinfection operations. The objective of this study was to evaluate the influence of light on the attachment and survival of Salmonella Typhimurium in lettuce (*Lactuca sativa* var *crispa*) leaves during storage at 4 ° C. The lettuce leaves were experimentally contaminated with a GFP-tagged *S.* Typhimurium suspension (10^8 CFU/mL) and incubated for 2 h at 4 ^oC for attachment, and then rinsed to remove unattached bacteria. The number of bacteria in the suspension was determined by plating on LB agar, incubated at 37 °C for 24 h. The leaves were stored in the dark (D) (0µE $m^{-2}s^{-1}$) or light (L) (100µE $m^{-2}s^{-1}$) for 72 h. Pieces from each lot of infected leaves were removed every 24 h and submitted to enumeration of *Salmonella* by plating on MLCB agar and to scanning electron microscopy SEM observations (Quanta 650FEG FEI), after treatment with 5% glutaraldehyde for 2 h, and washing with increasing concentrations of ethanol. After washing for removal the non-adherent cells, counts of *S.* Typhimurium in the leaves remained above 3 log CFU/mL, indicating adherence to the surface. Attachment was higher ($p<0,05$) in the leaves preserved in the dark (D) (4.01 CFU/mL) when compared to the ones in the light (3.06 CFU / mL). SEM showed that *S.* Typhimurium cells were located near and inside the open stomata and indicated possible formation of biofilm on the surface of the leaves after 24h. Study of the influence of environmental factors on attachment and internalization of pathogens has public health implications since internalized and adhered bacteria (biofilms) can escape disinfection and pose risks to the consumers.

Keywords: *Salmonella* Typhimurium, attachment, lettuce, scanning electron microscopy.

Acknowledgements: FAPESP, CNPq and Technological Characterization Laboratory.