Biofilm formation Salmonella Enteritidis on food contact surfaces

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Research hypothesis: Biofilms formed in food processing environments represent a long-term source of food contamination. Existence of bacterial biofilms in these environments may cause cross- and post- process contamination. The aim of the present study was to determine biofilm forming ability *Salmonella* Enteritidis on glass and stainless steel surfaces.
METHOD: Tests were performed with 15 isolates of *S.* Enteritidis (SE1-SE15). The glass and stainless steel coupons (1 x 1 x 0.2 cm) were sterilized and appointed separately into the recesses of polystyrene 12-well plate. Afterwards, 100 µL of bacterial suspension (~1-2 x 10^8 CFU/mL) was inoculated to surface of each coupon. Bacterial adhesion was provided during the 3h at 25/37°C. Subsequently, suspension was removed and coupons were washed with physiological saline, and submerged in 2 mL of Tryptone Soya Broth (TSB). Coupons were incubated for 48h at 25/37°C. Excess medium and non-adhered cells removed by mild pipetting with 3 mL of saline. Each coupon was placed in tube containing 1 mL of saline peptone solution. Detachments of bacteria were performed exposing tubes with coupons to low energy ultrasound for 3 minutes at 40 kHz, using ultrasound water bath. Afterwards, they were vortexed for 1 min at maximum speed and resuspended in 9 mL peptone saline solution. The number of cells that form biofilm was determined by a standard technique of colony counts on Tryptone Soya agar (TSA). Three coupons were analyzed for each tested isolates and the results were expressed as log CFU/cm².
RESULTS: The extent of adhesion ability varied among tested isolates depending on the tested temperatures and surfaces. The results showed that tested isolates produced significantly more biofilm at 25°C.
The weakest adherence ability to tested surfaces was shown by isolates SE3 and SE8. Adhesion assay on glass surfaces showed that adherence ability of tested isolates was ranged from 1.22 log CFU/cm² (SE3) to 2.48 log CFU/cm² (SE8) at of 25°C, and from 1.18 log CFU/cm² (SE3) to 1.88 log CFU/cm² at 37°C. Adherence ability these isolates to stainless steel surfaces was ranged from 1.21 log CFU/cm² (SE3) to 2.40 log CFU/cm² (SE8) at 25°C, and from 1.18 log CFU/cm² (SE3) to 1.98 log CFU/cm² at 37°C. Adhesion ability rest of tested isolates on glass surfaces was ranged from 3.64 log log CFU/cm² (SE10) to 7.0 log CFU/cm² (SE15) at 25°C, and from 3.06 log CFU/cm² (SE10) to 4.48 log CFU/cm² (SE7) at 37°C. Adhesion ability rest of tested isolates on stainless steel surfaces were ranged from 3.74 log CFU/cm² (SE10) to 7.36 log CFU/cm² (SE15) at 25°C, and from 3.04 log CFU/cm² (SE10) to 4.75 log CFU/cm² (SE7) at 37°C. Incubation temperature of 25°C ($p < 0.05$), with the exception of SE3 ($p > 0.05$), was more favorable to this ability. In contrast to the influence of temperature, tested isolates exhibited a greater propensity to adhere to stainless surfaces, but statistically significant differences were not found.
Within this research, the ability of SE isolates to colonize surfaces was demonstrated, particularly at ambient temperatures, which are common in food processing facilities. Therefore, future investigation should be based on establishing their prevention and eradication and as well as discovery of new biofilm control strategies.

Keywords: Salmonella Enteritidis, biofilm, glass surfaces, stainless surfaces