Salmonella enterica adhesion to different surfaces in the presence of *Echerichia coli* – Biofilm analyses by fluorescence *in situ* hybridization using a new peptide nucleic acid probe

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AIMS

Salmonella species have shown a great ability to adhere to solid surfaces and form biofilms. These may serve as pathogens reservoirs enhancing the risk of public health issues. In nature, biofilms are not usually formed by a single-species and the presence of other organisms may affect *Salmonella* population dynamics on surfaces. In this study, the *Salmonella enterica* serovar Enteritidis ability to adhere to different surfaces when in pure culture or co-cultured with *Escherichia coli*, was investigated.

METHODS

E. coli ATCC 25922 and *S.* Enteritidis ATCC 13076 suspension of ~ 1×10^8 total cells/ml in TSB were prepared. For each suspension (single and two species) 6 ml were added to each well of a 6-well tissue culture plate containing coupons of different materials. The plate was incubated at 24ºC ±2 and samples were taken with time (2, 4,6,24 and 48h). Cells were detached using a sonication step for cultivability assessment, total counts (DAPI) and *Salmonella* counts using a species-specific peptide nucleic acid (PNA). Cells were also visualized *in situ* by PNA- Fluorescence in situ hybridization (FISH) counterstained with DAPI. Total biomass was quantified by crystal violet (CV).

RESULTS

For all seven materials tested results were similar whatever the nature of the adhesion material. Typically *E.coli* outnumbered *S. enterica* in the adhered population for all materials, except for copper that presented similar number of cells and CFU's for both populations. Cultivability and CV data revealed that total adhesion in the two-species culture is faster than *S. enterica* adhesion in pure culture, occurring in the first hours and then stabilizing with time. The direct detection (PNA FISH) in biofilms presented some limitations for particular types of adhesion materials mainly related with the autofluorescence of the support material. This limitation was overcome by disrupting the biofilm sample followed by hybridization in suspension. Direct counts by PNA-FISH were compared with CFU´s counts and provided similar values.

CONCLUSIONS

The *S. enterica* co-inoculation with *E. coli* did not interfered with the total number of *Salmonella* cells adhered to each material. Nevertheless, *E. coli* was typically the outnumbered species. Comparing PNA-FISH counts with CFU´s confirmed that this method represents a reliable tool for mixed/natural biofilm study, allowing specific and direct detectionand providing spatial organization.