O158

Curli fimbriae and cellulose production are influenced by environmental conditions and affect biofilm formation of *Salmonella enterica* subsp. enterica serovar typhimurium

G.A.A. Castelijn[1], S. van der Veen[1], M.H. Zwietering[2], R. Moezelaar[3], T. Abee[1]

[1]TIFN and Wageningen University, Food Microbiology, Wageningen, [2]Wageningen University, Food Microbiology, Wageningen, [3]TIFN, Wageningen

Salmonella is the causative agent of salmonellosis. *Salmonella* is frequently encountered in food-processing environments, leading to contamination of food products. *Salmonella* is able to adhere to surfaces and subsequently form biofilms. A biofilm is a population of bacterial cells attached to a surface, embedded in an extracellular matrix. Biofilms cause problems in both industrial and medial settings, since they are generally hard to eradicate due to increased resistance against disinfection treatments or antibiotics. It is expected that the extracellular matrix contributes to the increased resistance of biofilms. Earlier studies have shown that curli fimbriae and cellulose are important components of the extracellular matrix of *Salmonella* biofilms. Transcription of the genes involved in curli fimbriae and cellulose production are positively regulated by the regulator CsgD. The expression of *csgD* is influenced by environmental signals. Thus, environmental conditions might influence the production of curli fimbriae and cellulose, and the question arises whether differences in biofilm forming capacity could be linked to differences in the activation of these components, and whether this can be linked to the origin of isolation.

In this study, we analyzed the biofilm forming capacity of 51 *S. Typhimurium* strains from different origin in TSB and 1/20 TSB at 25 °C and 37 °C, using the CV assay. The strain collection could be divided in three different groups. Group A strains, mainly clinical, outbreak-associated and retail product isolates, produced dense biofilms in both media at 25 °C, and in TSB also at 37 °C. Group B strains, mainly industrial isolates, only formed dense biofilms in 1/20 TSB at 25 °C, and group C strains showed little to no biofilm formation. Combining the results of the CV assay with enumeration of biofilm cells, suggests that biofilm formation as determined by the CV assay is dependent on both the number of biofilm cells and the extracellular matrix. Noticeably, the contribution of both factors is variable between different environmental conditions, with specifically biofilms cultured at 25 °C in 1/20TSB showing distinct composition and morphology. Therefore the contribution of the matrix components curli fimbriae and cellulose to biofilm formation was further assessed. The expression of genes encoding for the transcriptional activator CsgD, the major curli structural subunit CsgA and the post transcriptional regulator of cellulose production AdrA, was analyzed under different culture conditions in planktonic and biofilms cells, using quantitative real-time PCR. Our results showed that *csgA*, *csgD* and *adrA* are particularly induced during biofilm formation at 25 °C in 1/20 TSB. In addition, microscopic images of biofilms grown in 1/20 TSB at 25 °C showed high levels of calcofluor staining, which is an indicator for cellulose production, while only low levels of calcofluor staining was observed in biofilms grown in TSB.

In conclusion, we demonstrated that biofilm forming behavior is affected by environmental conditions and strains origin. In addition, it was shown that curli fimbriae and cellulose contribute specifically to biofilm production under low nutrient conditions at ambient temperatures and that other components are conceivably more important during biofilm formation at 37 °C and/or in nutrient-rich conditions by the group A strains.