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Regulation of biofilm formation in *Salmonella typhimurium* and *Escherichia coli* Nissle 1917

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ABSTRACT

Bacteria have the ability to grow in cell communities designated biofilms. This mode of growth is widespread and offers numerous advantages to the bacteria in terms of survival, persistence and propagation. Bacteria have developed different ways of building up a biofilm. Complex regulatory mechanisms control this sophisticated mode of growth in response to environmental conditions.

This thesis focuses on the regulation of biofilm formation by the food-borne pathogen *Salmonella enterica* serovar Typhimurium and the probiotic strain *Escherichia coli* Nissle 1917.

Commonly, species of the family of Enterobacteriaceae produce the biofilm extracellular matrix components cellulose and curli fimbriae at low temperature. The expression of cellulose and curli is activated by the transcriptional regulator CsgD. In this work, we demonstrated an altered pattern of biofilm regulation in *E. coli* Nissle 1917 (Paper I). Biogenesis of curli fimbriae was activated by CsgD at low temperature, while cellulose production at 28°C and 37°C did not require CsgD nor the di-guanylate cyclase AdrA. Cellulose production was, however, still dependent on the second messenger c-di-GMP. This regulatory pattern of cellulose and curli fimbriae production has been conserved in *E. coli* Nissle 1917 clonal isolates for more than 80 years implying biological significance. Production of cellulose mediated adhesion of *E. coli* Nissle 1917 to the gastrointestinal epithelial cell line HT-29 and to the mouse epithelium in vivo, thus possibly playing a role in colonization of the gut.

A characteristic of biofilm formation is cell heterogeneity. In *S. typhimurium*, expression of the master regulator CsgD was bistable during biofilm development (Paper II). Bistability led to task distribution, whereby the subpopulation of cells, which expressed high amounts of CsgD, was associated with microcolony formation and the production of cellulose.

CsgD expression is tightly regulated and responds to a variety of environmental conditions such as nutrient starvation and oxygen tension. Several global regulators contribute directly or indirectly to CsgD regulation. In this work, we identified novel factors involved in the complex CsgD regulation. Two lytic transglycosylases, MltE and MltC redundantly activated CsgD and rdar morphotype expression (Manuscript III). The absence of these two lytic transglycosylases could be partially compensated by the second messenger c-di-GMP. The chaperone Hfq and two Hfq dependent sRNAs, ArcZ and RyeB, also activated rdar morphotype expression by controlling the expression of CsgD (Manuscript IV). We demonstrated that ArcZ is a key regulator of biofilm formation. In addition, ArcZ played a role in the transition between sessility and motility and was involved in the timing of type 1 versus curli fimbriae surface attachment.

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