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Mechanisms in *Listeria monocytogenes* biofilm formation and disinfectant resistance

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The food-borne pathogen *Listeria monocytogenes* is frequently encountered in food processing facilities on food contact surfaces, on floors, and in drains, resulting in (cross-) contamination of food products. Several studies have shown that certain strains of *L. monocytogenes* can be present in food processing environments for years. Their presence and survival of disinfection treatments is expected to be dependent on their ability to form (stress resistant) biofilms. Since biofilms are generally more difficult to eradicate during disinfection treatments, the capability of *L. monocytogenes* to form biofilms poses a major concern for the food industry. Possible mechanisms involved in the increased resistance of biofilms to disinfectants are the restricted penetration of the biofilm, the slow growth rate of organisms in the biofilm, and the induction of resistance mechanisms in the biofilm. So far, little is known on the function of stress resistance mechanisms in biofilm formation and their resistance to disinfectants. For *L. monocytogenes* biofilms, two distinct morphologies have been identified. *L. monocytogenes* static biofilms on polystyrene and glass consists of a homogeneous layer, while on stainless steel *L. monocytogenes* biofilms consist of single attached cells or microcolonies. Static biofilms contain the small rod-shaped morphology, which is very similar to the morphology of planktonic cells. However, *L. monocytogenes* continuous-flow biofilms consist of ball-shaped microcolonies, which are surrounded by a dense network of knitted chains composed of elongated cells.

In this study, we investigated the role of the stress genes *recA*, which encodes the transcriptional activator of the SOS response, *sigB*, which encodes a major transcriptional regulator of the class II stress response genes, *hrcA*, which encodes the transcriptional regulator of the class I heat-shock response, and *dnaK*, which encodes a class I heat-shock response chaperone protein, in *L. monocytogenes* static and continuous-flow biofilm formation and/or their function in the resistance of biofilm cells to disinfectants. We showed that continuous-flow biofilm formation and not static biofilm formation is dependent on RecA and the SOS response. Using Q-PCR analyses, promoter reporters, and in-frame *recA* and SOS response deletion mutants, we showed that *recA* and the SOS response are activated during continuous-flow biofilm formation and that deletion of the SOS response gene *yneA*, which is involved in cell elongation during SOS response activation, results in diminished biofilm formation under continuous-flow conditions. Furthermore, Q-PCR and promoter reporter studies showed that *sigB*, *hrcA*, and *dnaK* are activated in static and/or continuous-flow biofilms. Biofilm formation studies using in-frame deletion mutants and complementation mutants showed that the presence of SigB, HrcA, and DnaK is required to obtain wild-type levels of both static and continuous-flow biofilms. Finally, disinfection treatments of planktonic grown cells and cells obtained from static and continuous-flow biofilms showed that SigB, HrcA, and DnaK are involved in the resistance of both planktonic cells and biofilms to the disinfectants benzalkonium chloride and peracetic acid. In conclusion, our study highlighted the impact of stress resistance mechanisms on biofilm formation and resistance of biofilms against disinfectants.