Identification and understanding the roles of biofilm formation-related genes in *Listeria monocytogenes* isolated from seafood

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This work is dedicated to my mother and father

Ihr gebt mir Wurzeln in die eine, und Flügel in die andere Hand und einen Kuss auf meine Stirn, der sagt mir: „Ich bin nicht alleine."
Dann legt ihr zwischen uns ein Band, sodass wir uns nicht verlieren, sagt ihr. Und dass ich gehen kann wenn ich will.
Und irgendwann geh ich raus. Aber hier draußen ist es so still, so ohne euch. Ihr seid nicht da wenn ich aufstehe, seid nicht da wenn ich schlafen gehe. Also schon, aber woanders und das ist nicht leicht.
Aber ich kann das. Und trotzdem fehlt ihr.
Auch wenn ihr mich nicht gefragt habt, gibt es da noch etwas, dass ich euch noch nicht gesagt hab. Ihr seid mein Ursprung, meine Insel, mein Vertrauen und mein Schatz. Mein Mund formt euer Lachen, mein Herz schlägt euern Takt.

Julia Engelmann

Abstract

Listeria monocytogenes is a foodborne pathogen that can lead to severe bacterial infections in immunocompromised people, the elderly and pregnant women and their unborn. Seafood is one of several contamination sources and as the seafood industry is of high economic value to New Zealand, this pathogen needs to be controlled. The main route for contamination is thought to be from biofilms in the seafood-processing environment and their persistence through cleaning and sanitation.

Persistent and sporadic strains of *L. monocytogenes* isolated from mussel-processing facilities were compared using phenotypic assays. Biofilm formation was greater for persistent strains compared to sporadic strains (30°C, 48h) using cell counts and crystal violet staining (CV). The persistent isolate 15G01 exhibited greatest biofilm formation and was therefore chosen to be studied for biofilm formation using transposon mutagenesis.

A screen of the transposon library for biofilm-forming ability using the crystal violet assay identified 27 genes to be associated with biofilm formation. Three low biofilm formers (33E11, 39G5, 44D3) and one high biofilm formers (34F11) were analysed with the fluorescent LIVE/DEAD stain and the scanning electron microscope revealing coccoid-shaped cells and long chains for 33E11 and 44D3, respectively.

The four mutants and a previously identified fifth (6B4) were investigated for their biofilm-forming ability, the surface characteristics of the cells and the influence of cations on biofilm formation. Three different biofilm formation assays were used to assess the composition of the biofilm. The CV assay was used to determine the whole biofilm mass, cell enumeration was applied to calculate viable cells in the biofilm and a formazan based assay (XTT) measured metabolic activity. All three assays showed a significant correlation, however, no correlation with cell surface characteristics was observed. Confocal laser scanning microscopy revealed a unique sandwich structure for the biofilm formed by 44D3, which has not been reported before, and was reversed at higher magnesium concentrations.

Magnesium influenced biofilm formation at a concentration of 5 mM resulting in enhanced biofilm formation for the wild-type and the mutant 44D3 and in reduced biofilm formation in 39G5 whereas calcium showed no gene-specific effect on biofilm formation.

The research presented in this study provides useful data for the prevention and control of biofilm formation by *L. monocytogenes* in seafood-processing plants.

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Declaration

The presented thesis is comprised of seven chapters. Chapters 3, 4, 5 and 6 are structured as manuscripts that have either been submitted and published or are to be submitted to peer-reviewed journals. Therefore, sections in the materials and methods are repeated in some chapters, however, results and discussion are unique to each chapter.

Contents

List of Publications

Nowak J., Cruz C., Visnovsky S., Palmer J., Fletcher G., Pitman A., Flint S. (in advanced preparation for submission). The *mgtB* mutant of the persistent *Listeria monocytogenes* 15G01 strain produces a unique biofilm sandwich structure which changes its phenotype upon addition of magnesium.

Nowak J., Cruz C., Visnovsky S., Palmer J., Fletcher G., Pitman A., Flint S. (in advanced preparation for submission). Biofilm formation of *Listeria monocytogenes* 15G01, a persistent isolate from a seafood-processing plant in New Zealand, is influenced by inactivation of multiple genes belonging to different functional groups.

Nowak J., Cruz, C., Tempelaars, M., Abee, T., van Vliet, A., Fletcher G., Hedderley, D., Palmer J., Flint S. (2017). Persistent *Listeria monocytogenes* isolates from mussel production facilities form more biofilm but are not linked to specific genetic markers. *International Journal of Food Microbiology*, Volume 256, 45-53

Nowak J., Visnovsky S., Pitman A., Cruz C., Fletcher G., Palmer J., Flint S. (Nov 2016). Magnesium and stress response play a vital role in biofilm formation of *Listeria monocytogenes.* NZMS Conference 2016, Christchurch, New Zealand (oral presentation).

Nowak J., Cruz C., Palmer J., Fletcher G., Flint S. (Nov 2016). Identification and understanding the roles of biofilm formation-related genes in *Listeria monocytogenes* isolates from seafood. NZMS Conference 2016, Christchurch, New Zealand (poster presentation).

Nowak, J., Cruz, C.D., Palmer, J., Fletcher, G.C., Flint, S. (2015). Biofilm formation of the *L. monocytogenes* strain 15G01 is influenced by changes in environmental conditions. *Journal of Microbiological Methods*, Volume 119, 189-195.

Nowak J., Cruz C., Fletcher G., Visnovsky S., Pitman A., Flint S., Palmer J. (Nov 2015). Identification of biofilm-related genes of *Listeria monocytogenes* isolated from seafood-processing premises. NZMS Conference 2015, Rotorua, New Zealand (poster presentation).

Nowak J., Cruz C., Fletcher G., Visnovsky S., Pitman A., Flint S., Palmer J. (Jun 2015). Identification of biofilm-related genes of *Listeria monocytogenes* isolated from seafood-processing premises. FEMS conference 2015, Maastricht, The Netherlands (poster presentation).

Nowak J., Cruz C., Fletcher G., Flint S., Palmer J. (Nov 2014). Optimizing methodology to better understand biofilm formation by *Listeria monocytogenes.* NZMS Conference 2014, Wellington, New Zealand (oral presentation).

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