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# Factors contributing to biofilm formation of *Yersinia enterocolitica*

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A thesis presented in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy in Food Technology

Massey University, Palmerston North, New Zealand



**MASSEY UNIVERSITY**  
**TE KUNENGA KI PŪREHUROA**  
**UNIVERSITY OF NEW ZEALAND**

**Haoran Wang**

December 2016

## Abstract

Biofilms of pathogenic bacteria are recognised as a threat to food safety. The aim of the present study was to investigate the potential of *Yersinia enterocolitica* to form biofilms in the pork processing environment and identify the resistance of these biofilms to sanitation. The biofilm formation by *Y. enterocolitica* was monitored at conditions simulating pork processing environment under daily cleaning routine using an impedance method established in this study. Results showed that *Y. enterocolitica* had the potential to form biofilm and become resistant to sanitation in a pork processing environment. An investigation into the factors influencing biofilm formation of *Y. enterocolitica* indicated that the  $\text{Ca}^{2+}$  ion increased the level of biofilm formation. In addition, the presence of the virulence plasmid pYV is essential for the biofilm  $\text{Ca}^{2+}$  response. Further analysis of the bacterial cell surface properties and extracellular polymeric substance (EPS) composition suggested that the pYV<sup>+</sup> cell surfaces are more negatively charged and more hydrophobic than the pYV<sup>-</sup> cells although no significant difference was observed with the addition of  $\text{Ca}^{2+}$ . The pYV<sup>+</sup> cells appear to produce more exopolysaccharide than the pYV<sup>-</sup> cells regardless of  $\text{Ca}^{2+}$  concentration.  $\text{Ca}^{2+}$  was able to increase the yield of extracellular DNA while the presence of pYV appeared to be dispensable in terms of extracellular DNA release. Analysis of cell wall protein revealed one protein expressed in the pYV<sup>+</sup> cells but absent in the pYV<sup>-</sup> cells.

## List of publications

**Wang H**, Tay M, Palmer J, Flint S (2016) Biofilm formation of *Yersinia enterocolitica* and its persistence following treatment with different sanitation agents. Food Control 73: 433-437.

**Wang H**, Palmer J, Flint S (2015) A rapid method for the nonselective enumeration of *Yersinia enterocolitica*, a foodborne pathogen associated with pork. Meat Science 113: 59–61.

## List of conference presentations

Flint, SH., **Wang, H.**, Palmer, J. (2016, November). *Yersinia enterocolitica* - factors contributing to biofilm formation. Presented at New Zealand Microbiological Society Conference. Christchurch, New Zealand.

Flint, SH., **Wang, H.**, Palmer, J. (2015, November). Influence of certain metal ions on the biofilm formation by *Yersinia enterocolitica*. Presented at New Zealand Microbiological Society Conference. Rotorua, New Zealand.

Flint, SH., Tay, M., **Wang, H.** (2015, October). The resistance of *Yersinia enterocolitica* biofilm to sanitizing agents under simulated food processing conditions. . Presented at 7<sup>th</sup> ASM Conferences on biofilm, Chicago, USA.

## Acknowledgements

First and foremost I would like to thank my chief supervisor Prof Steve Flint. It has been a great honour to be able to have Steve as my mentor in research and in life. I appreciate all his contributions of time, ideas, and funding to make my research experience productive and stimulating. It's his never-ending encouragement and trust that has helped me head through difficult times in my Ph.D. pursuit.

I also thank Dr Jon Palmer, my co-supervisor, for sharing his experience and expert knowledge during my research. Jon has provided me many valuable advices which helped me a lot during my thesis writing.

I am grateful for our lab members Mrs Anne-Marie Jackson, Mrs Julia Good and Mrs Kylie Evans for their help on my lab work. The group has also been a source of friendship which makes my lab time a joyful experience.

I would also like to acknowledge Ms Niki Murray at Manawatu Microscopy and Imaging Centre and Dr Dave Wheeler at New Zealand Genomics Limited for providing the Scanning Electron Microscopy and RNA-sequencing service.

Lastly, great thanks go to my parents and my younger brother for their love and encouragement. I am especially thankful for my husband and all his support in helping me muddle through ups and downs over the past few years even though he is far away in China.

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## List of abbreviations

eDNA: extracellular DNA

EPS: extracellular polymeric substances

ERL: enteric reference laboratory

ESR: environmental science and research

HIC: hydrophobic interactive chromatography

MJ: meat Juice

PCR: polymerase chain reaction

pYV: plasmid of *Yersinia* virulence

pYV<sup>+</sup>: pYV positive

pYV<sup>-</sup>: pYV negative

QAC: quaternary ammonium compounds

SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

SEM: scanning electron microscopy

TSA: trypticase soy agar

TSB: tryptic soy broth

Yops: *Yersinia* outer membrane proteins

Ysc: Yop secretion