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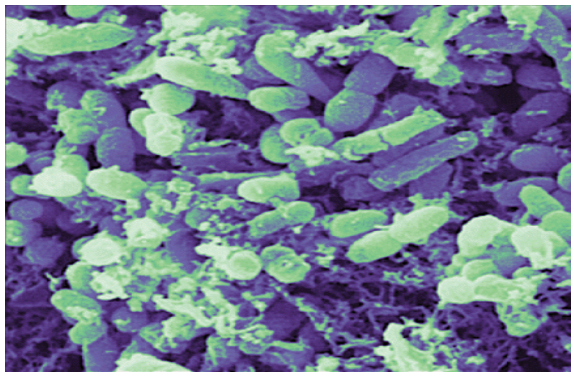
Genomics of gram negative food poisoning bacteria of animal origin



Key external stakeholders:
Food and in particular the pork sector, Regulators, FSAI

Practical implications for stakeholders:

Approximately 40% of Salmonella *Typhimurium* isolates examined readily attached to food contact surfaces and formed biofilms (bacterial populations on surfaces). In biofilms, *Salmonella* can persist for long periods of time, and pose a risk of contamination in food production. Research showed that particular genes and proteins needed to be expressed to allow formation of biofilms by *Salmonella*, and these could be targeted in development of new biocides.

Main results:

This project investigated the responses of food-borne bacteria to various stresses at the genomic and proteomic level. Research at Teagasc focused on the ability of *Salmonella* Typhimurium isolates to attach and persist as biofilms (bacterial populations attached to surfaces) in food production.

- Of the *Salmonella* Typhimurium isolates (n= 172) examined, which were recovered from the pork chain in Ireland or of human clinical origin, about 40% had the ability to form biofilms on stainless steel and plastic surfaces. Among clinical isolates 73% attached to PVC plastic compared to 53.3% of pork isolates. This indicates that the ability to persist on surfaces may be enhancing the transmission of *Salmonella* through the food chain to the consumer.
- *Salmonella* in biofilms formed at pH 5 showed increased expression of virulence genes *hilA* and *invA* compared to those from biofilms formed at neutral pH 7 indicating that acidic environments in food production plants may enhance the ability of *Salmonella* to cause food borne illness.
- In acidic environments, genes related to *Salmonella* motility i.e. flagella structures (*Flagellin*) were down regulated in cells from biofilms as compared to non surface attached (planktonic) cells. Genes related to cell-to-cell signaling and transport of exopolysaccharides across the outer membrane, were upregulated and needed for successful biofilm formation. Proteomic analysis also revealed that the switch from planktonic to biofilm status required up regulation of proteins associated with glycolysis, cell-to-cell signaling and protein transport.
- Therefore design of biocidal agents that specifically interfere with glycolysis and cell-to-cell signaling and that enhance flagella formation could help inhibit biofilm formation by *S*. Typhimurium in food processing facilities.

Opportunity / Benefit:

The data generated in this project gives a fundamental understanding on the persistence and biofilm formation by *Salmonella* on contact surfaces used in food production and could support industry in control of this pathogen and the development of novel targeted biocidal agents.

Collaborating Institutions:

University College Dublin

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1. Project background

Salmonella Typhimurium is a food-borne pathogen of importance to public health in Ireland and is a leading cause of human bacterial gastroenteritis. *S.* Typhimurium DT104 is frequently isolated from the pig/pork chain and this type of *Salmonella* is believed to have enhanced ability to attach to surfaces and to form biofilms (surface attached bacterial communities). When attached to surfaces and in biofilms, bacteria are believed to be better protected from anti-microbial controls and to persist for longer periods in the food environment. Cells existing in biofilms may exhibit different gene and protein expression when compared to non surface attached (planktonic) bacterial cells. The aim of the research was to gain a fundamental understanding, at a phenotypic, genomic and proteomic level, as to how *Salmonella* Typhimurium isolates recovered from the Irish pork chain attach to and persist as biofilms on food production surfaces.

2. Questions addressed by the project:

- How well do *Salmonella* Typhimurium DT104 isolates recovered from the Irish pork chain and from human illness, attach to food contact surfaces (PVC, stainless steel) and form biofilms?
- How is attachment and bioflm formation by *Salmonella* Typhimurium DT104 to surfaces impacted by acidic environments in food production environments?
- How are gene and protein expression in *Salmonella* Typhimurium DT104 impacted in biofilm formed at acidic pH?

3. The experimental studies:

- *Salmonella* Typhimurium isolates (n= 172) from the pork chain and of human clinical origin, were examined for their ability to attach to and form biofilms on stainless steel and plastic surfaces.
- Isolates were examined for presence of *Salmonella* genomic island 1 (SGI1), a 43 kb chromosomal genomic island that contains an antibiotic resistance gene cluster which confers multi-drug resistance to epidemic *S.* Typhimurium DT104. It has been proposed that the presence of SGI1 may also increase biofilm formation. *Salmonella* strains were examined for sections of SGI1 by PCR.
- *Salmonella* Typhimurium DT104 employs an acid tolerance response allowing it to adapt to acidic environments. The risk that these acid adapted cells pose to food safety could be enhanced if they also produce biofilms under acidic conditions. *Salmonella* were exposed to lactic acid and their ability to form biofilms on stainless steel was examined by looking at expression of selected genes related to regulation and virulence.
- A proteomics approach was used to examine differential protein expression in *S.* Typhimurium biofilm formed under acid conditions. Expression of selected proteins was examined by 2-D gel electrophoresis.

4. Main results:

Research focused on the ability of *Salmonella* Typhimurium isolates from the pork chain to attach and persist as biofilms (bacterial populations attached to surfaces) in food production.

- Of the *Salmonella* Typhimurium isolates (n= 172) examined of human clinical origin and the pork chain, 40% had the ability to form biofilms on stainless steel and plastic surfaces. Among clinical isolates 73% attached to PVC plastic compared to 53% of pork isolates. This indicates that the ability to persist on surfaces may be enhancing the transmission of *Salmonella* through the food chain to the consumer.

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- *Salmonella* genomic island 1 (SGI1) was present in isolates which were weak and strong biofilm formers indicating a weak association of this chromosomal genomic island with biofilm production
- *Salmonella* from biofilms formed at pH 5 showed increased expression of virulence genes *hilA* and *invA* compared to those from biofilms formed at neutral pH 7 indicated that acidic environments in food production plants are enhancing the ability of *Salmonella* to cause food borne illness.
- In acidic environments, genes related to *Salmonella* motility, (*Flagellin* which is involved in production of flagella structures) were down regulated in biofilms compared to non surface attached (planktonic) cells. Genes related to cell-to-cell signaling and transport of exopolysaccharides across the outer membrane, were up regulated and needed for successful biofilm formation. Proteomic analysis also revealed that the switch from planktonic to biofilm attached status required up-regulation of proteins associated with glycolysis, cell-to-cell signaling and protein transport.
- Therefore design of biocidal agents that specifically interfere with glycolysis and cell-to-cell signaling or that enhance flagella formation (motility) could help inhibit biofilm formation by *S*. Typmiurium in food processing facilities.

5. Opportunity/Benefit:

The data generated in this project gives a fundamental understanding on the persistence and biofilm formation by *Salmonella* on contact surfaces used in the food sector and could support industry in control of this pathogen and in the development of targeted biocidal agents.

6. Dissemination:

Main publications:

O'Leary, Denis, M. Mc Cabe, E., McCusker, M.P, Martins, M., Fanning, S. and Duffy, G. (2013) Microbiological study of biofilm formation in isolates of *Salmonella enterica* Typhimurium DT104 & DT104b cultured from the modern pork chain *International Journal of Food Microbiology* 5;161(1):36-43.

Martins. M., McCusker, M., McCabe, E., O'Leary, D., Duffy, G. and Fanning, S (2013). Study of the phenome(s) of Salmonella Typhimurium DT104 cultured from selected points across the pork production food chain - evidence of metabolic switching and implications for food safety. *Applied and Environ Micro*. 79(18):5437

Popular publications:

O'Leary, D., McCabe, E., McCusker, M., Martins, M., Fanning, S. and Duffy, G., (2012). Effect of lactic acid treatment on biofilm production and gene expression in the food-borne pathogen *Salmonella enterica* Typhimurium DT104. International Conference, Global Food Safety: Solutions for Today and Tomorrow. Crowne Plaza Hotel, Blanchardstown, Dublin, October 23 to 25th 2012. Pg. 94, ISBN 84170-591-8.

7. Compiled by: Geraldine Duffy, Kaye Burgess, Kieran Jordan and Declan Bolton

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