

COMPARATIVE EVALUATION OF PLANKTONIC AND BIOFILM
MODES OF GROWTH IN *SALMONELLA* TYPHIMURIUM

By

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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ABSTRACT

In this research, comparative studies of planktonic and biofilm modes of growth in *Salmonella enterica* serovar Typhimurium were undertaken. Chemical disinfectant studies were performed for both planktonic and biofilm *Salmonella* and biofilms at different ages. A gas chromatography coupled to mass spectrometry (GC-MS) metabolite profiling approach was used to evaluate metabolic variations between planktonic and biofilm *Salmonella* and biofilms at different ages.

Biofilm growth curves were generated to assess their ability to grow as a biofilm in the minimum biofilm eradication concentration (MBEC) assay system, and to achieve the most stable biofilm to carry out disinfectant susceptibility testing. Growth curves demonstrated that biofilms grown in nutrient poor conditions produced a stable biofilm over a seven-day growth period and as such, were used for susceptibility testing. Results showed that *Salmonella* biofilms were more resistant to disinfectants compared to planktonic *Salmonella*, but increasing the age of a biofilm did not increase resistance to disinfectants.

By using a GC-MS metabolomics approach, growth media were compared with supernatants from planktonic and biofilm cultures (extracellular metabolites); and biofilm cell extracts were compared with those from planktonic cells (intracellular metabolites). Principal component analysis (PCA) and spectral comparisons revealed differences between the overall metabolic profiles of planktonic and biofilm *S. Typhimurium*. The metabolic profiles of biofilms at different ages were subsequently obtained and evaluated. PCA and spectral comparisons revealed no significant

differences in intracellular metabolite profiles between biofilms of different ages. On the other hand, PCA analysis showed that the extracellular metabolite profiles of biofilms were different between the different ages of biofilm supernatant.

In conclusion, biofilms were more resistant to disinfectants compared to planktonic *Salmonella*, but this resistance was not increased in older biofilms. Metabolic analysis shows that there exist chemical differences between planktonic and biofilm modes of growth in *Salmonella*. Finally, there were differences in the extracellular metabolite profiles of biofilms at different ages, however such differences were not observed in intracellular metabolite profiles of *Salmonella* Typhimurium.

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ABBREVIATIONS

| | |
|------------|---|
| ABC | ATP-binding cassette |
| AgF | Thin aggregative fimbriae |
| AHL | Acyl-homoserine lactones |
| AI | Autoinducer |
| AI-1 | Autoinducer 1 |
| AI-2 | Autoinducer 2 |
| ATCC | American Type Culture Conditions |
| BapA | Biofilm-associated protein |
| BC | Benzalkonium chloride |
| CA | Citric acid anhydrous |
| CBD | Calgary Biofilm Device |
| CFU | Colony forming units |
| CG | Chlorhexidine gluconate |
| CLSM | Confocal Laser Scanning Microscopy |
| CsgD | Curlin sigma S-dependent growth |
| DNA | Deoxyribonucleic acid |
| DT | Definitive type |
| DPD | 4, 5-dihydroxyl-2, 3-pentanedione |
| ESCs | extended-spectrum cephalosporins |
| EPS | Extracellular polymeric substances |
| × <i>g</i> | times gravity |
| GC-MS | Gas chromatography coupled to mass spectrometry |
| LB | Luria-Bertani |

| | |
|-------------------------|--|
| LC | Liquid chromatography |
| Lux | <i>Vibrio fischeri</i> luminescence |
| <i>lsr</i> | luxS regulated |
| MBEC | Minimum biofilm eradication concentration |
| MDR | Multi-drug resistance |
| MIC | Minimum inhibitory concentration |
| MilliQ-H ₂ O | Milli-Q water |
| MSTFA | N-Methyl-N-(trimethylsilyl)trifluoroacetamide |
| NMR | Nuclear magnetic resonance |
| PCA | Principal Component Analysis |
| Pfs enzyme | 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase |
| PT | Phage type |
| QAC | Quaternary ammonium compound |
| QS | Quorum sensing |
| Rdar | red, dry and rough |
| <i>Rck</i> | resistance to complement killing |
| RNA | Ribonucleic acid |
| rpm | revolutions per minute |
| SAH | S-adenosyl-homocysteine |
| SahH | SAH hydrolase |
| SAM | S-adenosyl-L-methionine |
| SE | Standard error |
| SEM | Scanning electron microscopy |
| SdiA | suppressor of cell division inhibition A |
| SH | Sodium hypochlorite |

| | |
|-------------|--------------------------------------|
| <i>srgE</i> | <i>sdiA</i> -regulated gene <i>E</i> |
| SRH | <i>S</i> -ribosyl-homocysteine |
| VBNC | viable but non-culturable |
| XLD | Xylose lysine deoxycholate |

ORIGINAL PUBLICATIONS

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III Wong, H. S., Maker, G. L., Trengove, R. D., Fenwick, S. G., and O’Handley, R. M. (xxxx) Metabolic profiling of planktonic and biofilm *Salmonella* Typhimurium using gas chromatography mass spectrometry, submitted.

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