Copper Tolerance of

Listeria monocytogenes strain DRDC8

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Presentation of Figures and Tables

All figures and tables referenced in this thesis are placed at the end of relevant chapters. This has been done to minimise the impact of large numbers of figures and tables on document flow and as an aid to interpretation of results and discussion sections of each chapter.
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Abstract

Listeria monocytogenes is one of the most important food-borne pathogens due to the severity of the disease it can cause. While the virulence factors required for effective colonisation and infection of mammalian hosts have been well described, other genes may modulate disease persistence. For L. monocytogenes strain DRDC8, the ctpA gene encodes a copper transporting P-type ATPase that apparently maintains intra-cellular copper ion homeostasis (Francis & Thomas, 1997a) and is also required for persistent infection of the liver and spleens of mice (Francis & Thomas, 1997b). However, the distribution of this gene is apparently limited to non-clinically derived environmental L. monocytogenes isolates (Bell, 2002). This may be explained by carriage of ctpA on plasmid DNA (Bell, 2002). Based on predictions of function and proximity to the ctpA gene (pCT0020), ORFs pCT0017, pCT0018, pCT0019 and ctpA were identified as a putative a cop-like operon involved in copper ion transport in L. monocytogenes (Bell, 2002).

Southern hybridisation analysis was used to confirm that the ctpA gene is carried on plasmid pCT100 in strain DRDC8. In addition, evidence to suggest that ctpA was encoded by bacteriophage DNA was not obtained. Furthermore, sequence analysis of DNA flanking ctpA identified ORFs that encode polypeptide sequences similar to proteins involved in plasmid replication and other plasmid-associated functions. Mating experiments provided evidence to show that plasmid pCT100 is not conjugative. This suggested that lateral transfer of this plasmid between cohabitating organisms may be limited.

Sequence analysis of a 37.279 kbp region of plasmid pCT100 from L. monocytogenes strain DRDC8 (GenBank Accession U15554) showed this plasmid had regions of gene content and organisation similar to that of other characterised Listeria plasmids, particularly plasmid pLI100 from L. innocua CLIP11262 and plasmid pLM80 from L. monocytogenes strain 4b H7858. Gene’s common to these plasmids included those implicated in plasmid DNA replication, DNA transposition/insertion and heavy metal (cadmium) transport.

Sequence analysis of plasmid pCT100 also identified regions of DNA absent from other Listeria sequences. For example, a DNA region encoding a series of polypeptide
sequences similar to chromosomally-encoded proteins involved in copper transport in other Gram-positive bacteria was identified. The ORFs encoded by this region (pCT0017, pCT0018, pCT0019 and pCT0020 (ctpA), pCT0023, pCT0024, pCT0025, pCT0026, pCT0027) represent a novel cluster of genes implicated in copper homeostasis/tolerance that had not been previously described for other *Listeria* spp. PCR analysis was used to show that carriage of this copper gene cluster may be restricted to only some Australian *ctpA* positive *L. monocytogenes* isolates, typically of dairy and poultry origin.

In addition to these plasmid-encoded ORFs, PCR and sequence analysis identified a chromosomal ORF (*cutR*) also implicated in copper homeostasis/tolerance for strain DRDC8. *cutR* encodes a polypeptide similar to chromosomally-encoded copper-translocating P-type ATPases from other *Listeria* species.

The role of ORFs *cutR*, pCT0017, pCT0018 and pCT0019 in copper tolerance was assessed by comparison of the ability of wild type parent strain DRDC8 and variants containing independent mutations (pCT0017::erm, pCT0018::erm, pCT0019::erm or *cutR::erm*) to tolerate copper ion stress. The impact of loss of these genes (as a result of curing strain DRDC8 and *cutR::erm* derivatives of plasmid pCT100) on copper tolerance by DRDC8 was also examined. Minimal inhibitory concentration (MIC) and growth experiments showed that inactivation of *cutR*, pCT0018 or pCT0019, or removal of plasmid-encoded genes by curing DRDC8 of plasmid DNA, had a significant effect on copper tolerance. In addition, loss of plasmid DNA combined with disruption of *cutR* was shown to render cells completely incapable of growth in high levels of copper (14 mM CuSO₄). This data indicated that pCT0018, pCT0019 and *cutR* are involved in copper tolerance of *L. monocytogenes* strain DRDC8. MIC experiments also provided evidence to show that ORFs *cutR* and pCT0018 may play an additional role in tolerance to cadmium.

Interestingly, a *L. monocytogenes* mutant carrying an *erm* insertion within pCT0017 could not be constructed. However, evidence that showed that ORF pCT0017 encodes a CopY-like negative repressor protein directly implicated this ORF in copper tolerance. DNA gel shift experiments were used to show that pCT0017 protein binds to two ‘*cop box-like*’ nucleotide sequences located upstream of the pCT0017 translation start site. Binding occurs in a copper-dependant manner that is consistent with published models of CopY-like protein function. Thus pCT0017 protein may regulate expression of
ORFs pCT0017, pCT0018, pCT0019 and ctpA in a copper responsive manner. This is consistent with the view that these ORFs form a cop-like operon involved in copper homeostasis.

In conclusion, *L. monocytogenes* strain DRDC8 displayed an exceptional tolerance to high concentrations of copper ions. The data obtained suggested that both chromosomal and plasmid-encoded genes are involved in copper homeostasis/tolerance of DRDC8. This particular strain may have acquired multiple genes involved in copper tolerance from a co-habitating Gram-positive bacterium in response to exposure to high levels of copper within the environment. Given that strain DRDC8 is an Australian dairy isolate, these genes may provide a selective advantage for survival of other *L. monocytogenes* strains in associated environments.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Francesca York Bell and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Francesca Y Bell

November, 2010
Abbreviations

°°C° degrees Celsius
µg° microgram/s
µL° microlitre/s
µM° micromolar
× g° relative centrifugal force
aa° amino acid/s
AP° alkaline phoshatase
Amp° ampicillin
ATP° adenosine 5’-triphosphate
BHI° Brain Heart Infusion
bp° base pair
c.a.° circa = approximately
cf.° confer = compare
CFU° colony forming units
Cm° chloramphenicol
Ctp° copper transport protein
DIG° digoxigenin
DIG-11-dUTP° digoxigenin-11-uridine 5’triphosphate
DNA° deoxyribonucleic acid
dNTP° deoxyribonucleotide triphosphate
dsDNA° double stranded deoxyribonucleic triphosphate
DTT° dithiothreitol
EDTA° ethylene-diamine-tetra-acetic-acid disodium salt
Em° erythromycin
erm° erythromycin resistance gene
EtBr° ethidium bromide
g L⁻¹° grams per litre
h° hour/s
HCL° Hydrochloric acid
IPTG° isopropyl-β-D-thio-galactopyranoside
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</tr>
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<td>kanamycin</td>
</tr>
<tr>
<td>kbp</td>
<td>kilobase/s</td>
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RT  room temperature
s  second/s
SDS  sodium dodecyl sulphate
SLCC  Special Listeria Culture Collection
Sm  streptomycin
Sp  spectinomycin
spp.  species
SSC  standard saline citrate
TAE  tris-acetate EDTA buffer
TE  tris-EDTA buffer
UTP  uridine 5’triphosphate
UV  ultraviolet light
V  volt/s/
vol  volume/s
v/v  volume per volume
w/v  weight per volume
v/v  volume/volume
X-gal  5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside
X-pho  5-Bromo-4-chloro-3-indolyl-phosphate
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