Identification of Genetic Differences Between Strains of *Campylobacter jejuni*

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This dissertation is submitted for the degree of Doctor of Philosophy.

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

Emily Kay

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Abstract

The bacterial pathogen *Campylobacter jejuni* is known to cause a range of diseases from inflammatory diarrhoea to the autoimmune Guillain-Barré syndrome, although in some individuals infection with *C. jejuni* can be asymptomatic. The difference in outcome of the infection is likely to be the result of a number of factors including genetic differences between the infecting strains and susceptibility of the infected individual. As *C. jejuni* is known to be genetically variable, this project has involved the comparison of a number of unsequenced strains of *C. jejuni* against the sequenced strain 11168, in order to discover novel chromosomal sequences that may be responsible for the different phenotypes of these strains.

Four strains, representing a range of clinical outcomes and survival in different environmental niches were compared against the sequenced strain 11168 using a nylon macro-array based technique. This has resulted in the identification of 483 Kb of sequence containing 595 novel predicted genes within small-insert genomic libraries. Many of the novel predicted genes are associated with surface polysaccharide, flagellar biosynthesis and modification in addition to hypothetical genes. Also a number of genes identified were associated with restriction modification, metabolism and respiration. A few predicted proteins showed homology to genes associated with transposons, plasmid conjugation, chemotaxis and adhesion. Using this data 31 larger-insert BAC clones containing predicted genes of interest were identified and sequenced in order to determine the extent of these chromosomal islands. Within these sequences predicted genes have been identified that might be implicated in the distinct phenotypes of these strains.

A chromosomal tetracycline resistance determinant was discovered amongst remnants of transposon associated genes. A similar insert was found in two of eight

tetracycline resistant clinical isolates studied. This presents the possibility that a transposon may be responsible for disseminating tetracycline resistance in some strains of *C. jejuni*.

Two plasmids from one of the strains used in this study were sequenced. In one of the plasmids an inverting region was discovered and analysed, and the possibility that this is responsible for variable expression of a type IV secretion system was investigated.

Glossary of terms

aa	amino acid
ACT	Artemis Comparison Tool
AFLP	amplified fragment length polymorphism
AIDS	Aquired Immunodeficiency Syndrome
BAC	Bacterial Artificial Chromosome
BAP	Bacterial Alkaline Phosphatase
Bp	Base pair(s)
BSA	Bovine Serum Albumin
C	Cytosine
CDS	Coding Sequence
CDSC	Communicable Disease Surveillance Centre
CDT	Cytolethal Distending Toxin
dATP	2′-Deoxyadenosine 5′-triphosphate
dCTP	2′-Deoxycytidine 5′-triphosphate
DDW	double distilled water
dGTP	2′-Deoxyguanosine 5′-triphosphate
DMSO	dimethyl sulphoxide
dTTP	2′-Deoxythiamine 5′-triphosphate
EDTA	ethylene diamine tetra-acetic acid
FSA	Food Standards Agency
G	Guanine
GBS	Guillain-Barré syndrome
GGT	gamma-glutamyl transpeptidase
HLA	Human Lymphocyte Antigen
HR-MAS NMR	High-resolution magic angle spinning nuclear magnetic resonance
Id	Identity
IgG	immunoglobulin G
IPA	Invasion Protein Antigen
IPTG	isopropylthio-β-D-galactoside
IVS	Intervening sequence
Kb	kilobase(s)
LB	Luria-Bertani broth
LMP	Low Melting Point
LOS	Lipooligosaccharide

LPS	Lipopolysaccharide
LSHTM	The London School of Hygiene and Tropical Medicine
MFS	Miller Fisher Syndrome
MLST	Multilocus Sequence Typing
MSP	Maximal Segment Pairs
MW	Molecular Weight
PCR	Polymerase Chain Reaction
PEG	polyethylene glycol
PFGE	pulsed-field gel electrophoresis
PMSF	phenylmethylsulfonyl fluoride
RAPD	Randomly amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RM	restriction-modification
SDS	Sodium Dodecyl Sulphate
SOD	Superoxide Dismutase
SSD	Strain Specific DNA
ST	Sequence Type
T	Thymine
TE	Tris- EDTA
TLP	Transducer-like protein
TMAO	trimethylamine-*N*-oxide
TPS	Two Partner Secretion
TYE	Tryptone Yeast Extract
UV	Ultra Violet
WU-BLAST	Washington University-Basic Local Alignment Sequence Tool
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
YOP	*Yersinia* outer protein

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