Genetic elements within the genome of *Dichelobacter nodosus*

By

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I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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List of Abbreviations

a.a.	amino acids
b.p.	base pairs
CTAB	cetratrimethylammoniumbromide
dATP	adenosine triphosphate
dGTP	guanosine triphosphate
dH_2O	distilled water
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTPs	deoxyribonucleotide triphosphates
dsDNA	double stranded DNA
EDTA	ethylenediaminetetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
IS	insertion sequence
kb	kilobases
kbp	kilobase pairs
MW	molecular weight
NSW	New South Wales
nt.	nucleotides
ORF	open reading frame
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis

pfu	plaque forming units
PTA	phosphotungstic acid
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
SDS	sodium dodecyl sulphate
SLS	sodium lauryl sulphate
spp	species
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer ribonucleic acid
UA	uranyl acetate
UV	ultra violet
vap	virulence associated protein
vrl	virulence related locus
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside

Summary

The anaerobic bacterium *Dichelobacter nodosus* is the principal causative agent of ovine footrot. Analysis of the genomic DNA from virulent and benign strains of *D. nodosus* has identified regions of the genome which are present primarily in virulent strains and absent from most benign strains. These virulence-associated DNA regions, the *vap* (virulence associated protein) and *vrl* (virulence related locus) regions, have unknown functions and have both arisen by the integration of genetic elements into the bacterial genome.

In this study a genetic element, designated the *intB* element, adjacent to the *vap* regions has been partially characterised. The genes identified as part of the element are the integrase gene, *intB*, a regulatory gene, *regA*, and three genes of unknown function *gepA, B* and *C* (genetic element protein). The *intB* element is believed to have inserted into the same, or similar, site as the *vap* regions via the action of the *intB* gene product. The *regA* gene product is similar to a number of negative regulatory genes and *gepA* has been suggested as a positive regulator. The gene similarities of *intB*, *regA* and *gepA* suggest that the *intB* element may be an integrated bacteriophage or conjugative transposon-like element. The *gepB* and *gepC* genes have unknown functions.

To investigate the evolution of the *vap* regions in *D. nodosus* the sequences flanking *vap* regions 1 and 3 of strain A198 were investigated in strain C305, which does not contain the *vap* regions. These sequences were not adjacent in the genome of strain C305. Analysis of this intervening sequence has revealed a genetic element, designated the *intC* element, which consists of an integrase gene, *intC*, two genes of unknown function, *orf242* and *orf171*, *vapG"* and *H"* genes and a copy of the IS element, IS*1253*. Upstream of the *intC* element in strain C305 are the corresponding

ask and *tRNA-ser* genes to those located upstream of *vap* region 1 in strain A198. Remnants of the *intB* element are found downstream of the *intC* element. The *intC* element and the *vap* element may be unrelated, or may have diverged from a common ancestor. Alternatively, this region of the C305 genome may have contained both the *intC* element and the *vap* element, with subsequent loss of most of the *vap* element. The presence of a copy of IS*1253* in this region of C305 may account for the disruption of the adjacent *intB* element.

One or more of the genetic elements integrated into the *D. nodosus* genome may be prophages. To investigate this hypothesis, several strains of *D. nodosus* were treated with known bacteriophage-inducing agents. One of the strains of *D. nodosus* investigated was found to be lysogenic for an inducible bacteriophage. This bacteriophage, designated DinoHI, is a tailed phage consisting of an icosahedral head with a long non-contractile tail, claw-like base plate and a linear dsDNA genome. The DinoHI bacteriophage does not encode the previously identified integrated genetic elements. However, the prophage appears to lie adjacent to the right-hand end of the *vrl* region within the *D. nodosus* genome. DinoHI is able to adsorb to all *D. nodosus* strains tested, however, no lytic or lysogenic bacteriophage growth was detected. The DinoHI bacteriophage is the first native bacteriophage of *D. nodosus* to be characterised.

Table of Contents.

Publications arising from this thesis

Refereed papers

Bloomfield, G.A., Whittle, G., McDonagh, M.B., Katz M.E. and Cheetham, B.F. (1997). Analysis of sequences flanking the *vap* regions of *Dichelobacter nodosus*: evidence for multiple integration events and a new genetic element. *Microbiology* **143**: 553-562.

Unpublished papers

Whittle, G., Bloomfield, G.A., Katz, M.E. and Cheetham, B.F. Modulation of virulence by the integration of genetic elements into the genome of Dichelobacter nodosus, the causative agent of ovine footrot, (manuscript in preparation).

Bloomfield, G.A., Katz M.E. and Cheetham, B.F. Induction and characterisation of a temperate bacteriophage from *Dichelobacter nodosus*. (manuscript in preparation).

Conference abstracts

Garry A. Bloomfield, Margaret E. Katz and Brian F. Cheetham. Induction of a bacteriophage from *Dichelobacter nodosus*. Australian Society for Biochemistry and Molecular Biology Conference, Canberra, ACT. 1996.

Brian F. Cheetham, Garry A. Bloomfield, Gabrielle Whittle and Margaret E. Katz. Does the genome of *Dichelobacter nodosus* consist largely of mobile genetic elements ? 12th European Meeting on Bacterial Gene Transfer and Expression, Sienna, Italy. 1996.

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Refereed papers

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