Molecular analysis of the role of polynucleotide phosphorylase in the virulence of *Dichelobacter nodosus*

Suresh Kumar Athiappan Palanisamy

B.Sc., (University of Madras),

M.Sc.,(Bharathidasan University)

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“this is not the end;

this is not even the beginning of the end,

but this is perhaps the end of the

beginning"

WINSTON CHURCHILL.

Preface

This thesis describes research undertaken at the University of New England, Armidale, under the supervision of Associate Professor Brian F. Cheetham. I certify that the substance of this thesis has not been submitted as a part of another degree, and is not currently being submitted for any other degree or qualification. I further declare that all sources cited and any help received in preparing this thesis have been acknowledged.

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Suresh Kumar Athiappan Palanisamy

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Abbreviations

µg	microgram
µl	microliter
aa	amino acid
ADP	adenosine diphosphate
ATP	adenosine triphosphate
bp	base pair/s
CTAB	cetratri methyl ammonium bromide
dH_2O	distilled water
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTPs	deoxyriboculeotide triphosphate
dsDNA	double stranded DNA
EDTA	ethylene diamine tetra acetic acid
G6PD	glucose 6 phosphate dehydrogenase
GTP	guanosine triphosphate
IS	Insertion sequence
int	integrase
kan^R	kanamycin R
kbp	kilobasepair
MW	molecular weight
ng	nanogram
NSW	New South Wales
nt	nucleotide
ORF	open reading frame
PCR	polymerase chain reaction
PEP	Phosphoenol pyruvate
RNA	ribonucleic acid
RNase	ribonuclease
rRNA	ribosomal RNA
ser	serine
spp.	species
tetM	tetracycline M
tRNA	transfer RNA
UV	ultra violet
vap	virulence associated protein
vrl	virulence related locus
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside

Summary

Dichelobacter nodosus is the key causative agent of ovine footrot. *D. nodosus* strains display a range of virulence, from benign to virulent. In general, virulent strains secrete higher levels of thermostable proteases and have increased twitching motility compared to benign strains. Four integrated genetic elements have been identified, the *intA*, *intB*, *intC* and *intD* elements that integrate into *tRNA-ser* genes next to *pnpA* (polynucleotide phosphorylase) and *glpA* in *D. nodosus*.

PnpA acts as a global regulator of virulence in *Salmonella enterica* and GlpA is closely related to CsrA and RsmA, which act as global repressors of virulence in *Helicobacter pylori* and *Erwinia carotovora*. Whittle *et al.* (1999) proposed that genetic elements next to *glpA* and *pnpA* alter the expression of *glpA* and *pnpA*, thus regulating virulence in *D. nodosus*. The hypothesis that PnpA is a virulence repressor in *D. nodosus* was investigated by using a suicide plasmid containing two segments from *pnpA* flanking the *tetM* gene to produce *pnpA* knockouts in virulent and benign strains. Benign strains with *pnpA* knockouts had a significant increase in twitching motility, supporting the hypothesis that PnpA is a virulence repressor in *D. nodosus*. Complementation experiments confirmed that the increase in twitching motility was due to the *pnpA* knockout. Secreted proteases from virulent strains were found to be considerably more thermostable than proteases from benign strains, but the *pnpA* knockout in benign strains did not increase protease thermostability. A PnpA specific phosphorolytic activity assay showed that the virulent strains had a slightly lower level of PNPase activity than benign strains.

A qPCR assay was developed to measure mRNA levels from *pnpA* and *aprV2*, which encodes a thermostable protease. Differences in mRNA levels between strains were not large, and there was no correlation with virulence. However, there may be post-transcriptional regulation of *pnpA* and *aprV2* expression. Amino acid differences between both PnpA and AprV2 in benign and virulent strains were identified. Although these changes do not appear likely to alter the functions of PnpA and AprV2, their role in virulence should be investigated further.

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Publications associated with this thesis

Manuscript in preparation

Suresh Kumar, A. P., Fletcher. C., Hyman, J., Katz, M. E., and Cheetham, B. F. The C-terminal deletion of polynucleotide phosphorylase increases twitching motility, a virulence characteristic of *Dichelobacter nodosus*, the causative agent of ovine footrot.

Conference abstracts

Suresh Kumar Athiappan Palanisamy, Carrie Fletcher, Jessica Hyman, Margaret E Katz, and Brian F Cheetham. The putative global regulator gene of virulence, polynucleotide phosphorylase regulates the twitching motility: one of the virulence characteristics of *Dichelobacter nodosus*. Australian Society for Microbiology, Adelaide, South Australia. 2007.

Brian F Cheetham, Suresh K. A. Palanisamy, Carrie Fletcher, Dale Kenny, Warwick Locke, Jessica Hyman and Margaret E. Katz. Regulation of virulence gene networks by integration of mobile genetic elements. European Science Foundation – EMBO Symposium, Costa Brava, Spain. 2006.

Suresh Kumar Athiappan Palanisamy, Carrie Fletcher, Margaret E Katz, and Brian F Cheetham. The effect of polynucleotide phosphorylase knockouts on virulence characteristics of *Dichelobacter nodosus*. Australian Society for Biochemistry and Molecular Biology, Brisbane, Queensland. 2006.

Suresh Kumar Athiappan Palanisamy, Carrie Fletcher, Margaret E Katz, and Brian F Cheetham. The role of polynucleotide phosphorylase in the virulence of *Dichelobacter nodosus* the causative agent of footrot. Australian Society for Microbiology, Canberra, ACT, Australia. 2005.