

Investigation of Tail Fan Necrosis of Live-Held Southern Rock Lobsters

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Abstract

Tail fan necrosis (TFN) is a disease that affects southern rock lobsters during live-holding. The damage to affected tail fan uropods is seen as a major constraint in the development of a live-holding industry. A previous study has demonstrated that various *Vibrio* species are associated with diseased tissue (May, 2002. B.Sc. Honours Thesis, University of Adelaide). However, that study was restricted to an examination of TFN lesions that formed 8 weeks post infection for lobsters held under optimal growth conditions. Although damage to tail fan tissue by instruments contaminated by organisms isolated from TFN affected tissue was shown to result in formation of TFN-like lesions, the microbial community of lesions associated with TFN over time in terms of both the cultivable and non-cultivable communities was not identified. The extent of damage to tail fan tissue by bacteria and the response of lobster immune cells to infection was also not determined. Furthermore, the presence of potentially pathogenic *Vibrio* spp. within the diseased tissue was identified as a potential public health risk, particularly in food preparation facilities where live lobsters are handled. The work described in this thesis specifically examined the development and effect of TFN on the overall health of affected lobsters, as well a confirmation that the *Vibrio* spp. involved in establishment of TFN may represent a public health risk.

To answer these questions, a larger infection trial was set up. Uropod tissue of groups of lobsters were intentionally damaged with sterile instruments or instruments contaminated with a *Vibrio* spp. isolated from a TFN lesion. The lobsters were maintained in controlled environment aquaria and uropod tissue samples taken and subjected to microbiological, microscopic and molecular analysis.

Microscopic analysis of developing lesions demonstrated that several morphologically different bacterial cell types colonise the surface of TFN lesions. Bacteria involved in infection are essentially restricted to the surface of the lesions, but where significant damage to the uropod tissue occurs, these bacteria may invade the damaged tissue and penetrate deeper underlying tissue. Infection of tail fan tissue results in inflammation and concomitant loss of internal structure of the carapace and deposition of fibrous material within the soft tissue underlying the chitinous exoskeleton. In cases of severe inflammation, a central core develops within the fibrous tissue consisting of a

number of cell types, including hyaline cells, granulocytes and fibrocytes. However, there was no evidence of deep bacterial invasion into the underlying inflamed tissue.

Viable counts and identification of the bacteria associated with the diseased tissue demonstrated that the bacterial population of TFN lesions is dominated by *Vibrio* species. Whilst there was no significant increase ($P < 0.05$) in the total viable bacterial counts associated with the diseased tissue compared with healthy tail fan tissue, *Vibrio* species were isolated more frequently from tissue samples from uropods subjected to simultaneous damage and infection. 8% of bacterial isolates recovered from lesions were identified as *V. vulnificus* and 27% of isolates were identified as *V. parahaemolyticus*. Isolates of *V. vulnificus* displayed colony morphology consistent with pathogenic strains. Similarly, all isolates of *V. parahaemolyticus* were *tdh* negative, but 41% were *trh* positive. The majority of these species were able to express cytolysins capable of lysing CHO cells. This data indicated that vibrios responsible for establishment of TFN may have potential to cause human infections and therefore lobsters with TFN lesions should be regarded as a potential health risk to consumers. The majority of isolates of *Vibrio* spp. recovered from infected tissue expressed extracellular lipase and/or chitinase, and this indicated that these enzymes may enable *Vibrio* spp. to induce TFN in damaged tail fan tissue.

The predominance of *Vibrio* spp. associated with lesions was confirmed by analysis of amplicons representative of genes encoding 16S rRNA prepared from lesion tissue DNA extracts. This was achieved by sequencing randomly selected clones of amplicons and by use of Denaturing Gradient Gel Electrophoresis to separate amplicons according to nucleotide sequence diversity.

Unlike other crustacean shell diseases, TFN does not induce changes in serum protein levels, lead to significant bacteraemia or changes in the circulating haemocyte population. Furthermore, TFN has apparently little effect on the overall health of affected lobsters. This observation may explain the lack of mortality associated with this disease. Only a non-specific activation of lobster phenoloxidase in response to TFN was observed and only limited activation of phagocytosis of *Vibrio* spp. *in vitro* could be demonstrated. This data suggested that the lobster immune system is unable to respond to infection and may explain why the bacteria are able to induce persistent infection resulting in formation of TFN lesions. Nevertheless, localised melanisation surrounding the wound site induced

by carapace degradation products is able to restrict bacterial invasion into the haemolymph. The impact of TFN on appearance and consumer acceptance is dependent on the extent of damage caused by TFN. Minor lesions are resolved during moulting, whereas more severe lesions are maintained across more than one moult cycle.

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 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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Damian May

May, 2007

About Figures and Tables in this Thesis

The figures and tables in this thesis have been placed at the end of each relevant chapter or section.

Abbreviations

Ω	Ohms
$^{\circ}\text{C}$	degrees Celsius
μF	microfarad
μg	microgram/s
μL	microlitre/s
g	relative centrifugal force
A_{570}	absorbance at 570 nm
aa	amino acid/s
bp	base pairs
CFU	colony forming units
CHO	Chinese hamster ovary
d	days
DGGE	denaturant gradient gel electrophoresis
DNA	deoxyribonucleic acid
EDTA	ethylene-diamine-tetra-acetic-acid disodium salt
FCS	foetal calf serum
g L^{-1}	grams per litre
h	hour/s
HCl	Hydrochloric acid
IP	intra-peritoneal
IPTG	isopropyl- β -D-thio-galactopyranoside
Kb	kilobase/s
kDA	kilodalton/s
kg	kilogram/s
L	litre/s
LB	Luria Bertani broth
LD_{50}	lethal dose to 50% of the population
LPS	lipopolysaccharide

M	molar
mg	milligram/s
min	minute/s
mL	millilitre/s
mM	millimolar
nm	nanometres
NSW	New South Wales
NT	Northern Territory
OD	optical density
O/N	overnight
ONPG	o-nitrophenyl- β -D-galactopyranoside
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
<i>p.i.</i>	post infection
PO	phenoloxidase
ppA	prophenoloxidase activating enzyme
ppm	parts per million
proPO	prophenoloxidase
QLD	Queensland
RT	room temperature
SA	South Australia
SDS	sodium dodecyl sulphate
s	second/s
SEM	scanning electron microscopy
SI	super integron
spp	species
TAE	tris-acetate EDTA buffer
Tas	Tasmania
TCBS	thiosulphate citrate bile salt sucrose agar

<i>tdh</i>	thermostable direct haemolysin
<i>tlh</i>	thermo-labile haemolysin
<i>trh</i>	thermostable direct haemolysin-related haemolysin
TEM	transmission electron microscopy
TFN	tail fan necrosis
TTSS	type three secretion system
TVC	total <i>Vibrio</i> count
USA	United States of America
UV	ultraviolet light
VBNC	viable but non-culturable
VCR	<i>Vibrio cholerae</i> repeat
Vic	Victoria
vol	volume/s
v/v	volume per volume
<i>vvh</i>	<i>Vibrio vulnificus</i> haemolysin
WA	Western Australia
w/v	weight per volume

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