THE ROLE OF PODOVIRUS-LIKE BACTERIOPHAGE IN THE VIRULENCE OF *VIBRIO HARVEYI* STRAIN 47666-1

Thesis submitted by

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DECLARATION

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ABSTRACT

Two studies were conducted to demonstrate the transfer of virulence between the strains infected with phage VHPL and the same strains uninfected with the phage. The first study was to determine if a bacteriophage isolated from the more virulent strain (47666-1) would show the same virulence effects as bacteriophage VHML. The second study was to develop polyclonal and monoclonal antibodies specific to the toxic protein subunits of *V. harveyi* 47666-1. These antibodies were then used to detect this specific exotoxin from the previously naïve strains of *V. harveyi* infected with the phage VHPL.

SDS-PAGE analysis showed an up regulation and production of extracellular proteins in previously naïve *V. harveyi* strains receiving this phage compared with uninfected strains. Haemolysin assays indicated a significant increase ($P<0.001$) in both halo of clearing and colony diameter in bacteriophage-infected strains of *V. harveyi* compared to the same individual strains uninfected with the phage. However, siderophore production was not significant as all of the inducible strains did not respond positively on Chrome Azurol-S (CAS) agar. Chitin degradation, on the other hand, resulted in significantly greater zones of clearing ($P<0.001$) in strains infected with bacteriophage from *V. harveyi* 47666-1 than the same strains receiving no phage. In bath challenge assay, the results indicated that as a group, there was a significant difference in mortality rate among strains infected with bacteriophage from *V. harveyi* 47666-1 ($F=82.824$, $DF=9,40$, $P<0.001$) than strains of *V. harveyi* without the bacteriophage.

Polyclonal (PAbs) and monoclonal (MAbs) antibodies to the specific toxic subunits 45 and 55 kDa of *V. harveyi* 47666-1 were produced and used to detect specific toxic subunits from previously naïve strains of *V. harveyi* challenged with the phage. In western blot assay, PAbs produced only one specific toxin subunit having molecular weight of approximately 55kD in strains 30, 12 and 20 while no bands where obtained from strain 643. Two MAbs (3A1-9 and 5A2-1) were characterised and both detected toxic protein subunits in all naïve strains of *V. harveyi* infected with

the phage. However, cross reactions were observed in naïve strains 30, 12 and 20 uninfected with the phage.

In conclusion, the presence of bacteriophage VHPL from *V. harveyi* 47666-1 probably enhanced the virulence using assays with four naïve strains of *V. harveyi* as a model. There was significant up-regulation of proteins based on SDS-PAGE, up-regulation of haemolysins, chitinases and greater mortality to larvae of *P. monodon*. It is therefore suggested that the presence of this bacteriophage may either partly or fully confers virulence to *V. harveyi* strain 47666-1.

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LIST OF ABBREVIATIONS

A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
A_{600}	Absorbance at 600 nm
ABTS	2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid)
ACMM	Australian Collection of Marine Microorganisms
ANOVA	Analysis of variance
APS	Ammonium Persulphate
BDS	Bovine donor serum
BSA	Bovine serum albumin
CAS	Chrome Azurol-S
CFSE	Cell-free supernatant extract
CFU	Colony forming units
DAB	3,3'-diaminobenzidine tetrahydrochloride
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra-acetic acid
ELISA	Enzyme linked immunosorbent assay
FBS	Foetal bovine serum
HAT	Hypoxanthine-aminopterin-thymidine
HR	Halo ratio
HRPO	Horseradish peroxidase
HT	Hypoxanthine-thymidine
IP	Intraperitoneal
JCU	James Cook University
kb	Kilobases
kDa	Kilodalton
LD_{50}	Mean lethal dose
LSD	Least significance difference
MAbs	Monoclonal antibodies
mm	Millimeter
MW	Molecular weight

nm	Nanometer
NSW	New South Wales
OD	Optical density
OD_{600}	Optical density at 600nm
OPI	Oxaloacetate-pyruvate-insulin media supplement
PAbs	Polyclonal antibodies
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PVDF	Polyvinylidene fluoride
PYSS	Peptone, yeast, synthetic sea salt
RNA	Ribonucleic acid
SDS	Sodium-dodecyl-sulphate
TEM	Transmission electron microscopy
TEMED	Tetramethylethylenediamine
VHML	*Vibrio harveyi* Myovirus-like
VHPL	*Vibrio harveyi* Podovirus-like