INVESTIGATION INTO THE MORTALITIES OF LARVAL MUD CRABS, *Scylla serrata* AND METHODS OF CONTROL.

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October 2005

ABSTRACT

Pathogens and disease throughout the larval stage of the mud crab *Scylla serrata* are said to be responsible for preventing the establishment of a viable mud crab aquaculture industry within Australia.

Vibriosis has been demonstrated as the underlying cause for inconsistent survival of larvae of the mud crab *S. serrata* (Mann *et al*. 1998). Initial investigations of this study were directed at gaining an understanding of virulence factors of vibrios, namely siderophores, haemolysin, chitinases and bacteriophages. Haemolysin, chitinase and siderophore activity were assessed through observation on specifically prepared agars. Detection of a bacteriophage was conducted by mitomycin C induced growth curves. It was visibly evident that the LLD1 strain of *V. harveyi* had the greatest haemolysin production compared to the other strains. The *V. harveyi* strain 12 produced the least haemolytic activity. Colony and halo diameter of the strains were as a group, significantly different ($F=70.78, df=4, 100, P<0.0001$) and ($F=148.31, df=4, 100, P<0.0001$) respectively. When examining chitinase activity, strains 12 and LLB2 were the only isolates that did not produce an evident zone of clearance. Colony and halo diameter of chitinases of the above strains were as a group, significantly different ($F=41.46, df=5, 60, P<0.0001$) and ($F=118.03, df=5, 60, P<0.0001$) respectively. It was visibly evident that the selected probiotic isolate *V. harveyi* LLB1 had the greatest siderophore production compared to the other strains. Colony and halo diameter of siderophores of the strains were as a group significantly different ($F=22.79, df=4, 50, P<0.0001$) and ($F=172.97, df=4, 50, P<0.0001$) respectively. From the four isolates that were concentrated through ultracentrifugation and analysed by TEM, *V. harveyi* 642 was the only isolate to possess a bacteriophage.

The effectiveness of an environmental probiotic, referred to as the *V. alginolyticus* LLB2, was assessed on the survival of larvae of *S. serrata* when challenged with a virulent *V. harveyi* strain. The factors of the experimental trial, the bacterial isolate and concentration of bacteria, were significantly different as a group ($P<0.0001$) indicating that survival of zoea were affected by these factors. It was evident that there was a definite trend between increase in concentrations of bacteria and an increase in mortalities. It was also evident that treatments with *V. alginolyticus* LLB2 led to the

highest survival, which also happened to be at a concentration of 10^5 CFU ml^{-1}. Treatment consisting of *V. harveyi* LLD1 obtained the lowest survival at a concentration of 10^5 CFU ml^{-1}. Treatments that were inoculated at a cell density of 10^5 CFU ml^{-1} with the virulent *Vibrio harveyi* strains LLD1 and 642 and supplemented with the probiotic *V. alginolyticus* LLB2 had significantly higher survival rate (P>0.05) than the treatment inoculated with *V.harveyi* LLD1 and 642 alone. These results suggest that the probiotic *V. alginolyticus* LLB2 demonstrated a protective effect towards the larvae of *S. serrata* when challenged with a virulent *V. harveyi* isolate.

A histological investigation was carried out on the possible diseases associated with the larvae of *S. serrata*, and adult mud crabs. Cells within the hepatopancreas in both the larvae and adults showed nuclear pathology. Amorphous basophilic intranuclear inclusions, which were markedly hypertrophied were observed. These observations were similar to the initial surveys of the intranuclear bacilliform virus *Scylla* baculovirus virus (SBV) in adult *S. serrata* (Anderson and Prior 1992). From 15 batches of larvae that were surveyed only two batches of individuals were found to carry SBV. The prevalences of infected larvae were 32.26% and 52.17 %, respectively, where as 8% of 25 adult crabs from the Townsville region were infected.

Following the detection of an intranuclear bacilliform virus within the larvae of *S. serrata*, presumably SBV, an attempt was made to amplify the nucleotide sequence of this virus. Novel primer sets were designed from the protein-binding gene from WSSV which is said to be homologous to that of the insect baculovirus. Only the primer set BP2, BP2 5' – AAAAATGGTTGCCCGAAGCTC and BP2 5' – TGAGGAACGGCGACGGACAG-3' were successful in producing amplicons. When the relatedness of the amplicon sequences were compared to available databases using Basic Local Alignment Search Tool (BLAST), there were no significant phylogenetic matches.

This has been the first time the intranuclear bacilliform virus SBV has been identified in larvae of *S. serrata,* as well as the first partial sequence from SBV. The investigation of virulence mechanisms possessed by *Vibrio* sp. as well as the use of environmental probiotics in attempt to control vibriosis, will provide a sound basis for future studies in diseases in the mud crab *S. serrata*.

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ACMM	Australian collection of marine microorganism
BP	Base pair
BLAST	Basic Local Alignment Search Tool
CCEAD	Consultative Committee on Emergency Animal Diseases
CFU	Colony forming units
DAB	Diaminobenzidine Tetrahydrochloride
DNA	Deoxyribonucleic acid
DPI&F	Department of Primary industries and Fisheries.
ECP	Extracellular products
EDTA	Ethyldiaminetetraacetic acid
IBV	Intranuclear bacilliform virus
LA	Luminous agar
LBB	Luria bertani broth
LB	Luminous broth
LSD	Least significant difference
MARFU	Marine and Aquaculture Research Facility
OD	Optical density
PCR	Polymerase chain reaction
PYSS	Peptone yeast and sea salt
SBV	*Scylla* baculovirus
TEM	Transmission electron microscopy
UV	Ultra-violet
VHML	*Vibrio Harveyi* Myovirus Like
TBE	Tris Borate - EDTA
TE	Tris, EDTA buffer
TES	Tris, EDTA, NaCl buffer
TCBS	Thiosulphate-citrate-bile salts-sucrose
WSSV	White spot syndrome virus

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