Gene and Proteomic analysis of differentially expressed protein in hemocytes of giant fresh water prawn *Macrobrachium rosenbergii* infected with Infectious hypodermal and hematopoietic necrosis virus (IHHNV)

Alinejad, T1,2. Vejayan, J. 3, Othman, R.Y1,2 and Bhassu, S. 1,2

1. Genetics and Molecular Biology Div., Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

2. Centre for Research in Biotechnology for Agriculture, CEBAR, University of Malaya Kuala Lumpur, Malaysia

3School of Medicine and Health sciences, Monash University Sunway Campus, Jalan Lagoon Selatan, 46150 Subang Jaya, Selangor Darul Ehsan, Malaysia.

INTRODUCTION

Epizootic diseases cause huge mortality and economical loses at post larvae stages in freshwater prawn aquaculture industry. These prawns seem less susceptible to viral diseases except for Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV). During viral infection in prawns, hemocytes are the primary organ that shows immunological response within the early stages of infection. We applied proteomic approaches to understand differential expression of the proteins in hemocytes during the viral disease outbreak. To aid the goal, we collected *Macrobrachium rosenbergii* brood stocks from the local grow out hatchery which reported the first incidence of IHHNV viral outbreak during larvae stage. Primarily, application of the OIE primer targeting 389 bp fragments of IHHNV virus were used in identification of the infected and non-infected samples of the prawn breeding line. Analysis of two-dimensional gel electrophoresis showed specific down-regulation of Arginine kinase and Sarcoplasmic calcium-binding protein and up/down-regulation of Prophenoloxidase1 and Hemocyanin isoforms. These proteins were validated using semi quantitative RT-PCR and gene transcripts at mRNA level. These identified proteins can be used as biomarkers, providing a powerful approach to better understanding of the immunity pathway of viral disease with applications in analytic and observational epidemiology diagnosis. Proteomic profiling allows deep insight into the pathogenesis of IHHNV molecular regulation and mechanism of hemocyte in Freshwater prawns.

MATERIALS AND METHODS

Diagnostic Lab

- Sample collections
- Serum, DNA and Protein isolation and Quantification
- IHHNV infection detection and analysis

Wet Lab

- Two Dimensional Gel Electrophoresis (2-DE)
- Protein analysis and identification
- *Total RNA extraction and first strand cDNA synthesis Prophenoloxidase1 mRNA expression by qRT-PCR*

Dry Lab

- *Sample preparation & Solexa RNA-seq*
- Bioinformatics analysis

RESULTS

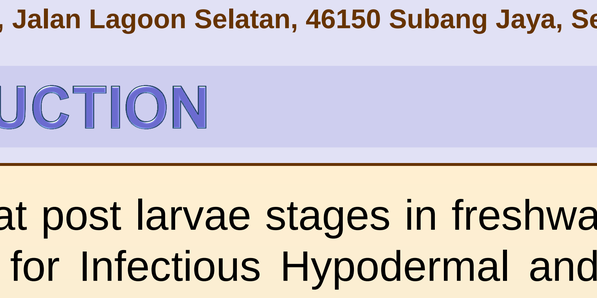

Figure 1. Detection of IHHNV in the infected Hemocyte.

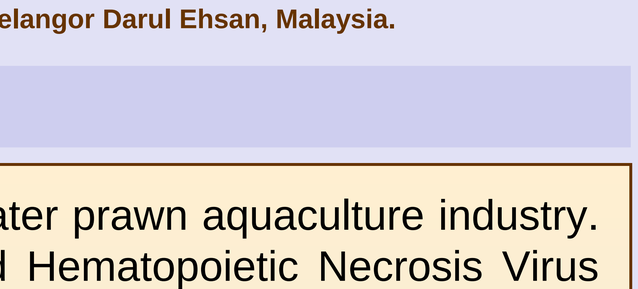

Figure3 Gene expression Prophenolixidase1 mRNA by qRT-PCR.

Figure 2. 2-DE profiles from Infected and non-infected Hemocyte
(A) indicate Infected group (B) indicate IHHNVNon-infected group

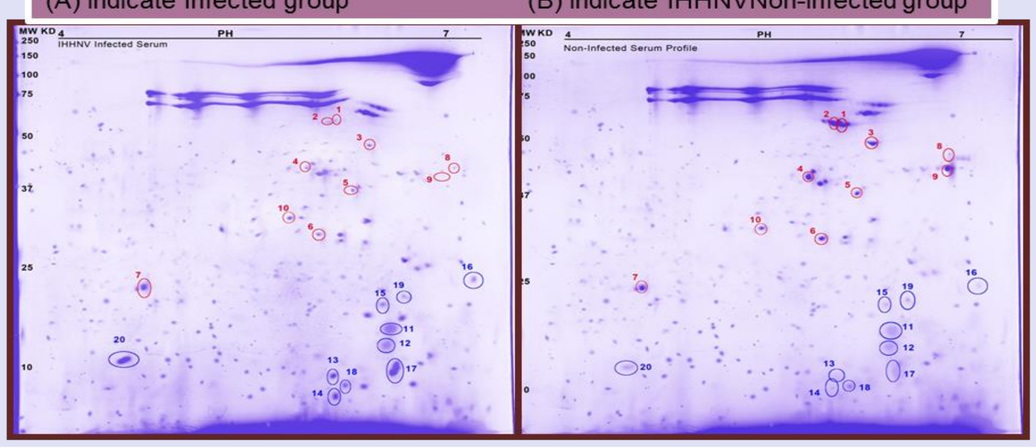

Table1: Identified proteins using PDQuest image analysis software Biorad and mas spectrometry

Sample Number	PI	Approximate M.W Mass (KD)	Result
1	5-6	50-77	Carbonic abhydrase2
2	5-6	50-75	NS
3	6-7	40-60	Enolase
4	5-6	30-40	Hemocyanin subunit
5	6-7	25-37	Hemocyanin subunit L
6	5-6	20-30	Hemocyanin subunit 1
7	4-5	15-20	Sarcoplasmic calcium-binding
8	7	37-50	Arginine Kinase1
9	7	37-50	Arginine Kinase1
10	5-6	20-30	NS

Sample Number	PI	Approximate M.W Mass (KD)	Result
1	6-7	10-20	Pro-phenoloxidase 1
2	6-7	10-20	Pro-phenoloxidase 2
3	6-7	10-20	Pro-phenoloxidase 3
4	6-7	8-15	Pro-phenoloxidase 1
5	6-7	15-20	Pro-phenoloxidase 4
6	6-7	15-25	Putative uncharacterized protein
7	6-7	10-15	Putative uncharacterized protein
8	6-7	15-20	Pro-phenoloxidase 1
9	4-5	15-20	Hemocyanin
10	4-5	10-20	NS

DISCUSSION

- This study generates significant information on *M.rosenbergii* immune system protein activity during IHHNV infection.
- Several differentially expressed protein identified are involved in various animal immune functions, such as antimicrobial, proteases and protease inhibitors, pattern recognition proteins, heat shock proteins, cell death, oxidative stress, blood clotting system, and prophenoloxidase system which was validate also at gene expression level.
- Results obtained provided a valuable insight into immunological mechanisms in *M.rosenbergii* and the role of the differentially expressed immune and environmental protein in response to IHHNV infection.

ACKNOWLEDGEMENTS

The authors would like to thank University Malaya (UM) for HIR funding, the Department of Biology, Faculty of science and Monash University Sunway Campus for all cooperation.

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