DISCOVERY OF IMMUNE RESPONSIVE PROTEINS IN IHHNV-INFECTED AND NON-INFECTED *MACROBRACHIUM ROSENBERGII*

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UNIVERSITI MALAYA
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Name of candidate: **TAHEREH ALINEJAD**

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Title of Thesis**: DISCOVERY OF IMMUNE- RESPONSIVE PROTEINS IN IHHNV-INFECTED AND NON-INFECTED *MACROBRACHIUM ROSENBERGII***

Field of Study: Genetic and Molecular Biotechnology

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ABSTRACT

The major problem in the prawn industry worldwide is acute epizootic diseases that are closely associated with explosive death among prawns which leads to economic losses in commercial aquaculture and posing threats to the indigenous wild stocks. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) was listed by OIE (Office of International Epizootics of World Organization for Animal Health) to be shown to have international significance in aquaculture. The study was conducted to understand the molecular responses of crustacean hemocytes to IHHNV infection. 2D-proteomic approach was used to investigate the differential expression proteins in hemocytes of infected and non-infected *M.rosenbergi* .Viral DNA screening has been done to identify infected (I) and non-infected (NI) samples by using IHHNV specific primer 309. The protein concentration of the serum to run on IPG-strip was determined using Bradford assay. To avoid protein degradation cup-loading method was used to perform IEF and consequently followed by 12.5% SDS-PAGE gel electrophoresis stained in silver nitrate. The protein identification has been done using MALDI TOF-TOF Mass spectrometry Statistical analysis was performed to infer the differential expressed proteins between the infected and non-infected prawns. Analysis revealed that 20 differentially expressed protein (10 up-regulated, 10 down-regulated) were identified. Finally, after database searches, these 20 proteins were categorized into 8 groups according to their function within the cell. These were proteins associated with energy production and catabolism (14%), cell function and physiology (14%), cell structure (5 %), ATP-buffering and environmental stress (5%), antioxidants (5%), calcium homeostasis (5%), oxygen transportation (19%) and immune system related protein (33%).

ABSTRAK

Masalah yang paling ketara dalam industri udang antarabangsa adalah penyakit epizootik yang dikaitkan dengan kematian udang pada suatu skala yang besar. Penyakit-penyakit ini mengakibatkan kerugian ekonomik dalam akuakultur komersil sambil mengancam stok-stok liar yang asli. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) telah disenaraikan oleh Office of International Epizootics of World Organization for Animal Health (OIE), sebagai sejenis penyakit bermasalah di seluruh dunia. Kajian ini dijalankan untuk memahami gerak balas hemosit udang terhadap infeksi IHHNV pada tahap molekul. Kaedah Proteomik-2D telah digunakan untuk menkaji perbezaan dalam ekspresi protein diantara udang yang dijangkiti oleh IHHNV dan udang yang tidak dijangkiti oleh IHHNV. Saringan DNA virus telah dijanakan untuk mengenal pasti udang yang dijangkit, dan udang yang tidak dijangkit dengan menggunakan primer 309 yang khas untuk IHHNV. Assay Bradford telah digunakan untuk menentukan konsentrasi protein serum yang diperlukan oleh strip-IPG. Untuk mengelakkan kemusnahan protein, kaedah cup-loading telah digunakan untuk IEF dan seterusnya diikuti oleh elektroforesis gel 12.5% SDS-PAGE yang menggunakan silver nitrat sebagai pengesan. Pengenalpastian protein sudah dijalankan dengan MALDI TOF-TOF Mass spectrometry. Analisa statistik telah digunakan untuk melihat perbezaan ekspresi protein diantara udang-udang yang dijangkiti dan yang tidak dijangkiti. Analisa ini menunjukkan kehadiran 20 protein yang diekspresi secara berbeza. Akhirnya, 20 protein ini telah diletakkan di dalam 8 kumpulan mengikut fungsi mereka di dalam sel hemosit. Kumpulan-kumpulan protein yang dikenal pasti adalah protein yang berkaitan dengan produksi tenaga dan katabolisme (14%), fungsi sel dan fisiology (14%), struktur sel (5%), penjagaan tahap ATP dan tekanan

persekitaraan (5%), antioksidan (5%), homeostasis kalsium (5%), pengangkutan oksigen (19%) dan protein yang berkaitan dengan system immune (33%).

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2-DE	2-dimensional electrophoresis
ACh	Acetylcholine
APS	Ammonium persulfate
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
DTT	Dithiothreitol
FBS	Fetal bovine serum
HCl	Hydrochloric acid
PBS	Phosphate buffer solution
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-Polyacrylamide gel electrophoresis
TEMED	Tetramethylethylenediamine
WHO	World health organization
SCP	Sarcoplasmic calcium binding protein
μg	microgram

µl	microlitre
nm	nanometer
U	Unit
V	Volt
v/v	volume by volume
w/v	weight by volume

MATERIALS AND INSTRUMENTATION

1. CHEMICALS AND REAGENTS

Acetic acid, Analar (England)

Acetone HPLC grade, Fisher Scientific (UK)

Acid alcohol

Acrylamide, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Agarose IEF, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Ammonium persulphate, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Albumin standard, Thermo Scientific (Rockford, USA)

Bis-acryamide, Bio-Rad Laboratories, Inc (Hercules, CA)

Bromophenol blue, Amersham Biosciences (Uppsala, Sweden)

Butan-1-ol, Merck (Darmstadt, Germany)

Cellulose MN300 plates (10 x 10 cm, 10 x 5 cm)

CHAPS, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Coomassie brilliant blue

Deionized water, Millipore

Disposable micro-pipettes, Eppendorf

DNA extraction Kit

DTT, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Ethanol, HmbG Chemicals

Ferric chloride

Filter paper, Whatman No.1, Whatman

Formaldehyde, Sigma-Aldrich (St Louis, USA)

Glycerol, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Glycine, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Hydrochloric acid

Immobiline drystrip, GE Healthcare Bio-Sciences (Uppsala, Sweden)

IPG Buffer, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Iodoacetamide, GE Healthcare Bio-Sciences (Uppsala, Sweden)

PhastGel Blue tablet (Coomassie R-350), GE Healthcare Bio-Sciences (Uppsala, Sweden)

Phosphoric acid (85%), Riedel-de Haen (Switzerland)

Silver nitrate, Analar (England)

Sodium carbonate, Ajax Finechem, (NSW, Australia)

Sodium chloride

Sodium dodecyl sulphate (SDS), GE Healthcare Bio-Sciences (Uppsala, Sweden)

Sodium nitrite, Merck (Darmstadt, Germany)

Sodium thiosulphate anhydrate, Ajax Finechem, (NSW, Australia)

TEMED, Amersham Biosciences (Uppsala, Sweden)

Tris-base, GE Healthcare Bio-Sciences (Uppsala, Sweden)

Urea, GE Healthcare Bio-Sciences (Uppsala, Sweden)

2. APPARATUS

Allegra® X-15R Series Benchtop Centrifuge, Beckman Coulter - for centrifuging cells prior to cell count

Beckman 340 pH temperature Meter, Beckman Coulter- was used to measure the pH value in phosphate buffer (7.0/7.4) preparation

Benchmark Plus Multiplate spectrophotometer, BioRad- was used to record photometric results for the MTS cytotoxicity assay.

CB-162 Stirrer hotplates, Stuart

Convection microwave NN-C2003S, Panasonic

Electronic analytical AB-S/FACT balance, Mettler Toledo

Electronic analytical PB-S/FACT balance, Mettler Toledo

Electrophoresis power supply FPS 601, GE Healthcare

Epson Expression 10000 XL scanner, Epson

ETTAN IPG PHOR III isoelectric focusing unit, GE Healthcare Bio-Sciences

Freeze dryer FDU-1100 machine, Eyela - was used to lypholyse the aqueus extracts

Freezer, Fisher & Paykel

Laminar flow cabinet ESCO class II type A2, Labculture

Microfuge® 18, Beckman Coulter- for centrifuging samples prior to isoelectric focusing

Milli-Q Integral Water Purification System, Millipore

Polymerase chain reaction Kit Promega

Refrigerator-freezer R-Z850 AM, Hitachi

SE 600 Ruby vertical electrophoresis system, GE Healthcare Bio-Sciences

Shaking water bath SBS40, Stuart

SWB analogue water bath, Stuart

Temperature control unit, Multitemp III, GE Healthcare

Ultra low freezer -152°C, Sanyo

Ultra low freezer -80°C Vip series, Sanyo

UV-1800 UV spectrophotometer, Shimadzu- was used to record photometric results for the Bradford protein quantification and Bradford dye-protein binding assays

Vortex mixer SA8, Stuart