UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF PROTEINS AND GENES AS POTENTIAL BIOMARKERS IN BROWN-MARBLED GROUPER (Epinephelus fuscoguttatus Forsskål) RESISTANCE TO Vibrio sp.

LOW CHEN FEI

IB 2014 14

IDENTIFICATION OF PROTEINS AND GENES AS POTENTIAL BIOMARKERS IN BROWN-MARBLED GROUPER (*Epinephelus fuscoguttatus* Forsskål) RESISTANCE TO *Vibrio* sp.

By

LOW CHEN FEI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

IDENTIFICATION OF PROTEINS AND GENES AS POTENTIAL BIOMARKERS IN BROWN-MARBLED GROUPER (*Epinephelus fuscoguttatus* Forsskål) RESISTANCE TO *Vibrio* sp.

By

LOW CHEN FEI

November 2014

Chair: Mariana Nor Shamsudin, PhD
Faculty: Institute of Bioscience

The gram-negative marine bacterium, *Vibrio* sp. has frequently been identified as the causative pathogen responsible for the infectious disease vibriosis in the marine aquaculture industry. This disease is one of the major challenges facing brown-marbled grouper aquaculture, causing fish farmers globally to suffer substantial economic losses. In this study, several experiments were conducted using a range of methodologies to identify proteins and genes that are immune response-related upon *Vibrio* infection in brown-marbled grouper. Serum proteome profiles from two-dimensional gel electrophoresis were compared between infected grouper and control grouper after 4 hours of pathogen challenge. Differentially expressed proteins were then identified by MALDI TOF. The serum proteins that were highly expressed during early *Vibrio* infection of grouper were putative apolipoprotein A-I, natural killer cell enhancement factor and lysozyme g. The transcriptome of brown-marbled grouper spleen was studied by RNA-sequencing using Next Generation Sequencing technology. Gene expression in grouper spleen was compared between the infected grouper and control grouper. A total of 4162 unigenes were up-regulated in infected grouper, and 4988 unigenes were down-regulated. Gene ontology classification showed 338 differentially expressed genes were involved in immune system processes. Cell killing and antioxidant activity class have highest percentage of differentially expressed unigene of 34.48% and 37.74% respectively. Up-regulated unigenes in the cell killing class included transporter-associated with antigen processing 2, and cytotoxic and regulatory T cell protein. KEGG pathway annotation identified eight immune-related pathways and also seven non-immune response-related pathways that were significantly enriched in differentially expressed genes. Among the most abundantly up-regulated unigenes, four unigenes were found to be novel and were not annotated in any of the database. These novel unigenes warrant further identification and characterization of its function in immune response of grouper against pathogens. Lastly, brown marbled grouper fingerlings observed for seven days after experimental infection with *Vibrio*

parahaemolyticus determined grouper susceptible to infection, with these fish having skin lesions ≥ 5mm. Grouper that were resistant to infection had no observable skin lesion. Skin lesion specimens observed under the scanning electron microscope revealed disintegration of skin around the lesion, and presence of bacterial cells under high magnification of 6000X. Serum proteome profiles were compared between the resistant and susceptible grouper by two-dimensional gel electrophoresis. Putative parvalbumin beta-2 subunit I, alpha-2-macroglobulin, nattectin and immunoglobulin light chain were identified to be differentially expressed in resistant grouper. In summary, resistance of grouper to bacterial infection involved complex mechanisms consisting of different pathways with distinct methods of activation and regulation. Genes and proteins altered in these pathways are potential markers to identify *Vibrio* resistant grouper as well as targets for immunomodulation and disease prevention through vaccination. It could therefore be concluded that putative parvalbumin beta-2 subunit I, alpha-2-macroglobulin, nattectin and immunoglobulin light chain are among the important proteins participating critically in disease resistance mechanism in grouper, which are over-expressed to function collectively, thus contributing to the resistance of grouper to *V. parahaemolyticus* infection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGENALAN PROTEIN DAN GEN YANG BERPOTENSI SEBAGAI PENANDA BIO DALAM IKAN KERAPU (*Epinephelus fuscoguttatus* Forsskål) RINTANG TERHADAP *Vibrio* sp.

Oleh

LOW CHEN FEI

November 2014

Pengerusi: Mariana Nor Shamsudin, PhD
Fakulti: Institut Biosains

Bakteria marin gram-negatif, *Vibrio* sp. sering dikenalpasti sebagai patogen yang menyebabkan penyakit berjangkit yang dikenali sebagai vibriosis dalam industri akuakultur marin. Penyakit ini adalah salah satu cabaran utama yang dihadapi dalam akuakultur ikan kerapu, menyebabkan penternak ikan di seluruh dunia mengalami kerugian ekonomi yang besar. Dalam kajian ini, beberapa reka bentuk eksperimen yang merangkumi pelbagai kaedah telah dijalankan untuk mengenal pasti protein dan gen yang berkaitan dengan gerak balas imunisasi ikan kerapu terhadap jangkitan *Vibrio*. Profil proteome serum dari elektroforesis gel dua dimensi telah dibandingkan di antara kerapu kawalan dan kerapu yang dijangkiti pathogen selepas empat jam. Protein yang diekspres lain dari kawalan telah dikenalpasti melalui kaedah MALDI TOF. Protein serum yang meningkat secara mendadak semasa jangkitan awal *Vibrio* dalam ikan kerapu ialah apolipoprotein A-I, factor perangsangan sel pembunuh semula jadi dan lisozim g. Transkriptom limpa ikan kerapu dikaji melalui penjujukan RNA dengan menggunakan teknologi penjujukan "next generation". Gen dalam limpa kerapu telah dibandingkan antara kerapu kawalan dan kerapu terjangkit. Sebanyak 4162 unigen yang diekspres telah meningkat dalam kerapu terjangkit, manakala 4988 unigen yang telah menurun. Klasifikasi gen ontologi menunjukkan 338 gen yang diekspres secara berbeza adalah terlibat dalam proses sistem imun. Kelas sel pembunuh dan aktiviti anti-pengoksidaan telah menunjukkan peratusan paling tinggi dalam gen yang diekspres secara berbeza iaitu 34.48% dan 37.74%. Dalam kelas sel pembunuh, unigen yang ekspresi telah meningkat termasuk "transporter-associated with antigen processing 2" dan "cytotoxic and regulatory T cell protein". Anotasi rangkaian KEGG mengenalpasti bahawa lapan rangkaian KEGG yang berkaitan dengan keimunan, dan tujuh rangkaian KEGG yang tidak berkaitan dengan keimunan telah diperkaya secara ketara dengan gen yang diekspres secara berbeza. Antara unigen yang ekspresi telah meningkat, empat unigen dikenalpasti sebagai novel dan tidak dianotasi dalam pangkalan data. Unigen yang novel ini perlu dikaji lebih mendalam untuk

mengetahui fungsi unigen ini dalam gerak balas keimunan ikan kerapu terhadap patogen. Ikan kerapu yang dijangkiti *Vibrio parahaemolyticus* secara eksperimen dan diperhatikan selama tujuh hari menunjukkan kerapu yang mudah terdedah kepada jangkitan mempunyai luka di kulit yang berukuran ≥ 5mm. Kerapu yang rintang jangkitan tidak mempunyai sebarang luka di bahagian kulit. Spesimen kulit yang terluka diperhatikan di bawah mikroskop imbasan elektron menunjukkan perpecahan kulit di sekitar luka, dan kehadiran sel-sel bakteria dapat diperhatikan di bawah kuasa pembesaran setinggi 6000X. Profil proteome serum telah dibandingkan antara kerapu yang rintang terhadap jangkitan dan kerapu yang mudah terdedah kepada jangkitan oleh elektroforesis gel dua dimensi. Parvalbumin beta-2 subunit I, alpha-2-macroglobulin, nattectin dan rantai ringan imunoglobulin telah dikenalpasti diekspres secara berbeza dalam kerapu yang rintang terhadap jangkitan. Secara ringkasnya, kerapu yang rintang terhadap jangkitan bakteria melibatkan mekanisme yang kompleks yang terdiri daripada pelbagai rangkaian proses biologi yang berbeza dengan kaedah yang berbeza dari segi pengaktifan dan pengaturannya. Gen dan protein yang dikawal atur secara berbeza dalam rangkaian proses biologi ini adalah penanda bio yang berpotensi untuk digunakan dalam mengenalpasti kerapu yang rintang terhadap jangkitan *Vibrio* serta digunakan dalam imunomodulasi dan terapi dalam vaksin. Kesimpulannya, Parvalbumin beta-2 subunit I, alpha-2-macroglobulin, nattectin dan rantai ringan imunoglobulin merupakan protein yang penting yang terlibat secara kritikal dalam mekanisma rintang terhadap jangkitan dalam ikan kerapu, yang mana ekspresinya telah meningkat dan berfungsi bersama justeru menyumbang kepada daya rintang terhadap jangkitan *V. parahaemolyticus* dalam ikan kerapu.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deep gratitude to my supervisory committee, Prof. Dr. Mariana Nor Shamsudin (Chairperson), supervisory committee members: Assoc. Prof. Dr. Maha Abdullah, Prof. Dr. Fatimah Md. Yusoff, and Dr. Chee Hui Yee, for their constant and thorough supervision and guidance throughout the entire period of this project. Without the help and support from the supervisory committee, this project may not have been completed with fulfilling expectations.

Sincere appreciation is extended to all seniors and staffs in the Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia for their immeasurable supports, assistance, and encouragement.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mariana Nor Shamsudin, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Maha Abdullah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Fatimah Md. Yusoff, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Chee Hui Yee, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:		Signature:	
Name of Chairman of Supervisory Committee:	Mariana Nor Shamsudin, PhD	Name of Member of Supervisory Committee:	Maha Abdullah, PhD
Signature:		Signature:	
Name of Member of Supervisory Committee:	Fatimah Md. Yusoff, PhD	Name of Member of Supervisory Committee:	Chee Hui Yee, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

VNN	Viral nervous necrosis
TDH	Thermostable direct haemolysin
TRH	TDH-related haemolysin
NGS	Next-Generation Sequencing
SNP	Single nucleotide polymorphism
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
FAO	Food and Agriculture Organization
RAS	Recycle aquaculture systems
SGIV	Singapore grouper iridovirus
RPS	Relative percentage survival
ROS	Reactive oxygen species
PRRs	Pattern recognition receptors
LPS	Lipopolysaccharide
PAMPs	Pathogen associated molecular patterns
CLRs	C-type lectin receptors
MHC	Major histocompatibility complex
MALT	Mucosa-associated lymphoid tissue
GALT	Gut-associated lymphoid tissue
SALT	Skin-associated lymphoid tissue
GIALT	Gill-associated lymphoid tissue
2-DE	Two-dimensional gel electrophoresis
PMF	Peptide mass fingerprinting
PBS	Phosphate buffered saline
CFU	Colony forming unit
Ppt	Parts per thousand
MS-222	Tricaine mesylate
IPG	Immobilized pH gradient
FPKM	Fragments Per kb Per Million Fragments
NKEF	Natural killer cell enhancement factor
nr	Non redundant
KEGG	Kyoto Encyclopedia of Genes and Genomes
COG	Clusters of Orthologous Group
GO	Gene Ontology
SEM	Scanning electron microscope

CHAPTER ONE

INTRODUCTION

Fish is one of the major protein sources that contributes a relatively high percentage of animal protein to the human diet. However, due to rapid population growth that increases the demand for fish protein, the present fish catch is insufficient to compensate the increasingly high market demand for fish. This has promoted the expansion of the aquaculture industry. High market value marine fish is being cultured extensively in parallel with the rapid expansion of the aquaculture industry. Brown marbled grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775) is one of the high market value marine fish that is well received in the industry. However, high incidence of disease outbreaks has been reported with intensive culture, leading to huge economic losses in the industry. Viral diseases such as viral nervous necrosis (VNN) have been reported in several cultured grouper species included *E. coioides*, *E. fuscoguttatus* as well as *E. bruneus*, and the geographic distribution is worldwide including in Asia (Japan, Taiwan, Indonesia, Brunei Darussalam), North America (United States of America) and Europe (France, Italy, United Kingdom) (Nagasawa *et al.*, 2004). Meanwhile, several gram negative *Vibrio* species have been frequently reported as the causative agents of a bacterial disease known as vibriosis. Vibriosis has been reported in cultured *E. coioides*, *E. tauvina* and *E. malabaricus* in Brunei Darussalam, Malaysia, Taiwan, Indonesia and Thailand (Nagasawa *et al.*, 2004).

Aquaculture methods include extensive farming, semi-intensive farming and intensive farming. Among the different culture methods, intensive farming is the most common way that is being practiced in most aquaculture industry. The intensive culture techniques include land-based intensive flow-through farming, recirculation aquaculture systems, and cage farming. Application of intensive culture techniques which includes high stocking density of fish has led to high organic content, low aeration and increased pollution which have sustained the multiplication of pathogens including bacteria and viruses in the culturing system. The aquatic environment that contains very high concentrations of these pathogens promotes outbreak of diseases. Use of agricultural antibiotics in fish farming and immune-suppression in fish caused by water pollution are among the factors that contribute to the outbreak of diseases (Kai 1993). Vibriosis, a common infectious disease frequently reported in the aquaculture industry is caused by strains of Gram-negative bacteria, collectively called *Vibrio*. *Vibrio* is widely distributed in the marine environment. Among the *Vibrio* species, *Vibrio parahaemolyticus* and *Vibrio cholera* are important pathogenic agents for aquatic animals, and *Vibrio alginolyticus* is also frequently identified as a disease-causing pathogen. Rapid expansion of the aquaculture industry and the increase in the intensity of mariculture has expanded the list of *Vibrio* species causing fish diseases. The outbreaks of vibriosis in aquaculture have led to huge economic losses

globally. Thus, strategies to improve culture conditions such as selective breeding are proposed to produce new strains of fish that are highly resistant to disease pathogens, inclusive of *Vibrio*.

The pathogenicity of *V. parahaemolyticus* has been reported to be due to its ability to produce haemolysins, a chemical substance that exhibit beta-haemolysis on high-salt blood agar (Noriea *et al.,* 2010). Haemolysins cause haemorrhagic septicaemia in marine fish, leading to mortality in cultured fish populations (Wong & Leong 1990). Previous studies showed that the majority of genes contributing to the virulence of *V. parahaemolyticus* include thermostable direct haemolysin (TDH), TDH-related haemolysin (TRH) and thermolabile haemolysin (Iida *et al.,* 1998; McCarthy *et al.,* 1999). The manifestation of *V. parahaemolyticus* infection in fish appears as tail rot, red spots on the head, swollen and necrotic intestine (Alcaide *et al.,* 1999; Shruti & Soumya 2012) as well as lesions on the body (Wong & Leong 1990). Other commonly observed signs included anaemia, ascetic fluid, petechial haemorrhages on the muscle wall and accumulation of liquid in the air bladder (Shruti & Soumya 2012). The early stage of *V. parahaemolyticus* infection is commonly marked by the appearance of red spots on the skin surface, which develop in size until a circular to oval-shaped, deep haemorrhagic ulcer is noticeable, exposing the skeletal musculature (Sankar *et al.,* 2012). The colonization and interaction of bacteria with the fish host is a complex process, and variation in disease severity is expected. These well-defined signs allow distinction of disease-susceptible from disease-resistant variants and are important in the subsequent study of the mechanisms of infection in fish.

A robust immune response in fish is the main mechanism of defense to resist infections. Mediators of the innate and adaptive immune responses consists of a large number of proteins, which function in a variety of mechanisms to restrict the invasion, and prevention of systemic spread of the pathogen that could progress into lethal infection (Wu *et al.,* 2004; Lauren & Hao 2007). The effectiveness of fish immune response against invasion by a pathogen determines the infection outcome. Recent progress has indicated the potential in development of marker assisted selection strategies in fish breeding schemes. Many of these include mediators of immune response. These studies have also revealed the complexities in pathways and vast number of molecules involved. Therefore, further investigation using genome-wide techniques may be helpful to determine the mechanisms of bacterial infection in fish due to specific resistance mechanisms that are peculiar to the fish species.

De novo transcriptome sequencing by Next-Generation Sequencing (NGS) technology is a suitable platform to rapidly identify differentially expressed genes on a large scale and also facilitates functional studies. Transcriptome sequencing provides information of global gene expression profiles, in

addition to the discovery of novel genes and single nucleotide polymorphisms (SNPs), as well as the assembly of full-length genes (Huang *et al.*, 2011; Vera *et al.*, 2008; Emrich *et al.*, 2007). To date, the genome sequence of *Epinephelus fuscoguttatus* is still unavailable. The application of NGS in the study of brown marbled grouper infected with Vibrio may identify markers associated with the immune system and this woud be useful for further studies in identification of disease resistant variants. On the other hand, study of grouper immune response upon bacterial infection through a proteomic approach identifies the proteins of interest and their regulation. Expression of proteins is regulated according to physiological needs, and their concentration may not be represented at mRNA level. Not all expressed genes are translated into proteins, but depend on the rate of translation and also the rate of mRNA degradation. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) provides higher gel resolution and is thus, suitable for comparative analysis of total proteome profile, integrated with spectrometric and bioinformatics approaches.

Hypothesis

Experimental infection of brown marbled grouper with *Vibrio* will identify resistant grouper expressing differential proteins compared to grouper that are susceptible to *Vibrio* infection. These differentially expressed proteins can be identified through comparative two-dimensional gel electrophoresis. In terms of genes expression, transcriptome sequencing of grouper spleen will identify differentially expressed genes in response to *Vibrio* challenge.

Specific objectives

- ~ To isolate and identify differentially expressed proteins in serum proteome profiles of infected grouper and *Vibrio* resistant grouper.
- ~ To identify differentially expressed genes in spleen transcriptome profiles of *Vibrio*-infected grouper.
- ~ To identify potential biomarkers of disease resistance in grouper.

REFERENCES

Alcaide E., Amaro C., Todoli R. & Oltra R. (1999) Isolation and characterization of Vibrio parahaemolyticus causing infection in Iberian toothcarp Aphanius iberus. Diseases of Aquatic Organisms 35, 77–80

Alexander JB, Ingram GA (1992) Noncellular nonspecific defense mechanisms of fish. Annual Review of Fish Diseases 2, 249-279

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of doublestranded RNA and activation of NF-[kappa]B by Toll-like receptor 3. Nature 413, 732–738

Ali M, Brian AW, Kenneth MC, Lorian S and Barbara W (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature Methods 5, 621-628

Amatruda JF, Patton E (2008) Genetic Models of Cancer in Zebrafish, in: Jeon, K. W., (Ed.), International Review of Cell and Molecular Biology, Academic Press, San Diego, CA, 1–34

Aranishi F, Nakane M (1997) Epidermal proteases of the Japanese eel. Fish Physiology and Biochemistry 16, 471-478

Avnimelech Y, Kochva M, Diab S (1994) Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israeli Journal of Aquaculture-Bamidgeh 46, 119-131

Baeuerle PA, Baltimore D. (1996) NF-kappa B: ten years after. Cell 87, 13–20

Balebona M.C., Andreu M.J., Bordas M.A., Zorrilla I., Morinigo M.A. & Borrego J.J. (1998) Pathogenicity of Vibrio alginolyticus for cultured gilt-head sea bream (Sparus aurata L.). Applied and Environmental Microbiology 64, 4269–4275

Bayne C.J. & Gerwick L. (2001) The acute phase response and innate immunity of fish. Development and Comparative Immunology 25, 725–743

Bayon Y, Ortiz MA, Lopez-Hernandez FJ, Gao F, Karin M, Pfahl M, Piedrafita FJ. (2003) Inhibition of IkappaB kinase by a new class of retinoid-related anticancer agents that induce apoptosis. Molecular and Cellular Biology 23, 1061–1074

Beck G, Habicht GS (1996) Immunity and the invertebrates. Scientific American, 275:60e3

Bergljót M (2006) Innate immunity of fish (overview). Fish and Shellfish Immunology 20, 137-151

Best JD, Alderton WK (2008) Zebrafish: an in vivo model for the study of neurological diseases. Neuropsychiatric Disease and Treatment 4, 567–576

Binoy R, Jorge MOF, Christopher MAC, Viswanath K, Jan HWMR, Monica FB (2011) Proteome reference map of the skin mucus of Atlantic cod

(Gadus morhua) revealing immune competent molecules. Fish and Shellfish Immunnology 31, 224-231

Blomhoff R, Blomhoff H.K. (2006) Overview of retinoid metabolism and function. Journal of Neurobiology 66, 606-630

Bowden T.J., Butler R., Bricknell I.R. & Ellis A.E. (1997) Serum trypsin-inhibitory activity in five species of farmed fish. Fish and Shellfish Immunology 7, 377–385

Braceland M, Bickerdike R, Tinsley J, Cockerill D, Mcloughlin MF, Graham DA, Burchmore RJ, Weir W, Wallace C, Eckersall PD (2013) The serum proteome of Atlantic salmon, Salmo salar, during pancreas disease (PD) following infection with salmonid alphavirus subtype 3 (SAV3). Journal of Proteomics 94, 423-436

Bugajska-Schretter A., Grote M., Vangelista L., Valent P., Sperr W.R., Rumpold H., Pastore A., Reichelt R., Valenta R. & Spitzauer S. (2000) Purification, biochemical, and immunological characterization of a major food allergen: different immunoglobulin E recognition of the apo- and calcium-bound forms of carp parvalbumin. Gut 46, 661–669

Cerenius L. & S€oderh€all K. (2004) The prophenoloxidaseactivating system in invertebrates. Immunological Reviews 198, 116–126

Chang SF, Ngoh-Lim GH, Kueh LFS, Qin QW, Seng EK, Sin YM (2002) Initial investigations into two viruses isolated from marine food fish in Singapore. Veterinary Record 150, 15–16

Chen J, Li C, Huang R, Du FK, Liao LJ, Zhu ZY, Wang YP. (2012) Transcriptome analysis of head kidney in grass carp and discovery of immune-related genes. BMC Veterinary Research 8, doi:10.1186/1746-6148-8-108

Chen J, Shi YH, Li MY, Ding WC, Niu H. (2008) Molecular cloning of liver angiotensinogen gene in ayu (*Plecoglossus altivelis*) and mRNA expression changes upon *Aeromonas hydrophila* infection. Fish and Shellfish Immunology 24, 659-662

Chen Y., Zhang Y.X., Fan T.J., Meng L., Ren G. & Chen S.L. (2006) Molecular identification and expression analysis of the natural killer cell enhancing factor (NKEF) gene from turbot (Scophthalmus maximus). Aquaculture 261, 1186–1193

Chu TW (1996) Grouper culture in Taiwan. Proceedings of the workshop on aquaculture of coral reef fishes and sustainable reef fisheries, Kota Kinabalu, Sabah, Malaysia, 6–10 December; 1996

Coffman R.L., Seymour B.W.P., Lebman D.A., Hiraki D.D., Christiansen J.A., Shrader B., Cherwinski H.M., Savelkoul H.F.J., Finkelman F.D., Bond M.W. & Mosmann T.R. (1988) The role of helper T cell products in mouse B cell differentiation and isotype regulation. Immunological Reviews 102, 5–28

Danayadol Y, Direkbusarakom S, Supamattaya K (1995) Viral Nervous Necrosis In Brown-Spotted Grouper, Epinephelus malabaricus, Cultured in Thailand. In: Shariff, M., Arthur, J.R., Subhasinghe, P.

(Eds.), Diseases in Asian Aquaculture II. Fish Health Section. Asian Fisheries Society, Manila, 227–233

Danilova N, Bussmann J, Jekosch K, Steiner LA (2005) The immunoglobulin heavy-chain locus in zebrafish: identification and expression of a previously unknown isotype, immunoglobulin Z. Nature Immunology 6, 295–302

Diaz M. & Casali P. (2002) Somatic immunoglobulin hypermutation. Current Opinion in Immunology 14, 235–240

Diggles BJ, Adlard RD (1997) Intraspecific variation in Cryptocaryon irritans. Journal of Eukaryotic Microbiology 44, 25–32

Du Pasquier L (2001) The immune system of invertebrates and vertebrates. Comparative Biochemistry and Physiology – Part B, 129:1e15

Edson K.I., Marcio J.F., Lidiane Z.G., Erica M.M.C., Evilin N.K., Alexandra A.C., Karina R.B., Monica L.F. & Carla L. (2012) Role of interplay between IL-4 and IFN-c in the regulating M1 macrophage polarization induced by Nattectin. International Immunopharmacology 14, 513–522

Edwin H.B. (1981) Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. The Journal of Infectious Diseases 143, 325–345

Ellis A.E. (2001) Innate host defense mechanisms of fish against viruses and bacteria. Development Comparative Immunology 25, 827–839

Emrich SJ, Barbazuk WB, Li L, Schnable PS (2007) Gene discovery and annotation using LCM-454 transcriptome sequencing. Genome Research 17, 69-73

Evans DL, Leary JH, Jaso-Friedmann L (2001) Nonspecific cytotoxic cells and innate immunity: regulation by programmed cell death. Developmental and Comparative Immunology 25, 791-805

FAO (1989) Aquaculture methods and practices: A selected review. FAO Fisheries and Aquaculture Department online. Rome

FAO (2010) Cultured aquatic species information programme, Epinephelus coioides. FAO Fisheries and Aquaculture Department online. Rome

FAO (2011) World aquaculture 2010. FAO Fisheries and Aquaculture Department. Technical Paper. No. 500/1. Rome, FAO. 105

Franz V., Adriana B., Alin C., Rodolfo A. & Margarita I.C. (2007) Apolipoprotein A-I, and antimicrobial protein in Oncorhynchus mykiss: evaluation of its expression in primary defence barriers and plasma levels in sick and healthy fish. Fish and Shellfish Immunology 23, 197–209

Fu N, Wang Q, Shen HL (2013) De Novo assembly, gene annotation and marker development using Illumina paired-end transcriptome sequences in celery (Apium graveolens L.). PlosONE 8(2): e57686. Doi:10.1371/journal.pone.0057686

Fukuda Y, Nguyen HD, Furuhashi M, Nakai T (1996) Massmortality of cultured seven band grouper, Epinephelus septemfasciatus, associated with viral nervous necrosis. Fish Pathology 31, 165–170

Ganassin RC, Bols NC (1996) Development of long-term rainbow trout spleen cultures that are haemopoietic and produce dendritic cells. Fish and Shellfish Immunology 6, 17-34

Gao L, He CB, Liu XG, Su H, Gao XG, Li YF, Liu WD (2012) The innate immune-related genes in catfish. International Journal of Molecular Sciences 13, 14172-14202

Genciana T, Salvatore P, Tonina R, Elena P, Marco S, Maria FA (2014) Proteomic profiling of sea bass muscle by two-dimensional gel electrophoresis and tandem mass spectrometry. Fish Physiology and Biochemistry 40, 311-322

Gillis J.M., Thomason D., Lefevre J. & Kretsinger R.H. (1982) Parvalbumins and muscle relaxation: a computer simulation study. Journal of Muscle Research and Cell Motility 3, 377–398

Giuseppe S (2013) Functional aspects of fish lymphocytes. Developmental and Comparative Immunology 41, 200-208

Graham HC, Wolf CR, Valerie MM and John AC. (1990) Changes in hepatic xenobiotic-metabolising enzymes in mouse liver following infection with *Leishmania donovani*. Molecular and Biochemical Parasitology, 41, 17-24

Greenberg S, Grinstein S. (2002) Phagocytosis and innate immunity. Current Opinion in Immunology 14, 136-145

Hansen JD, Landis ED, Phillips RB (2005) Discovery of a unique Ig heavy-chain isotype (IgT) in rainbow trout: implications for a distinctive B cell developmental pathway in teleost fish. Proceedings of the National Academy of Sciences USA 102, 6919–6924

Hardard_ottir I., Gr€unfeld C. & Feingold K.R. (1994) Effects of endotoxin and cytokines on lipid metabolism. Current Opinion in Lipidology 5, 207–215

Heizmann C.W. (1984) Parvalbumin, an intracellular calcium binding protein; distribution, properties and possible roles in mammalian cells. Experientia 40, 910–921

Helen D and Martin FF (2005) Shark immunity bites back: affinity maturation and memory response in the nurse shark, Ginglymostoma cirratum. European Journal of Immunology 35, 936-945

Heppell J. and Davis H.L. (2000) Intramuscular injection of DNA vaccines in fish. Methods in Molecular Medicine 29, 99-103

Honjo T. & Habu S. (1985) Origin of immune diversity: genetic variation and selection. Annual Review of Biochemistry 54, 803–830

Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. (1993) Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 260, 547-549

Huang YH, Huang XH, Yang Y, Cai J, Ouyang ZL, Cui HC, Wang PR, Qin QW (2011) Transcriptome analysis of orange-spotted grouper (Epinephelus coioides) spleen in response to Singapore grouper iridovirus. BMC Genomics 12, 556-567

Ignasi F, Joaquin A, Joan C (2010) Fish proteome analysis: Model organisms and non-sequenced species. Proteomics 10, 858-872

Iida T., Park K.S., Suthienkul O., Kozawa J., Yamaichi Y., Yamamoto K. & Honda T. (1998) Close proximity of the tdh, trh and ure genes on the chromosome of Vibrio parahaemolyticus. Microbiology 144, 2517–2523

Irene S, Zhang YA, Sunyer JO (2011) Mucosal immunoglobulins and B cells of teleost fish. Developmental and Comparative Immunology 35, 1346-1365

Irwin D.M. & Gong Z. (2003) Molecular evolution of vertebrate goose-type lysozyme genes. Journal of Molecular Evolution 56, 234–242

Iseli C, Jongeneel CV, Bucher P. (1999) ESTScan: a program for detecting, evaluating, and reconstructing potential coding regions in EST sequences. Proceedings of the International Conference on Intelligent Systems for Molecular Biology 99, 138-148

Jacob EC, Wamdaogo MG, Antoine S, Alphonse T, Kenneth DV, N'Fale S, Brian PL (2010) De Novo transcriptome sequencing in Anopheles funestus using Illumina RNA-seq technology. PlosONE 5(12): e14202. Doi:10.1371/journal.pone.0014202

Jane H.P., Erika H., Gong Y.Q., Aleksey S. & Tracey S. (2009) A physiological function for apolipoprotein (a): a natural regulator of the inflammatory response. Experimental Biology and Medicine 234, 28–34

Jayne ET and Nina I (2009) The role of CYP26 enzymes in retinoic acid clearance. Expert Opinion on Drug Metabolism and Toxicology 5(8), 8775-886

Jollès P. (1969) Lysozymes: a chapter in molecular biology. Angewandte Chemie 8, 227–294

Jutras I, Desjardins M. (2005) Phagocytosis: at the crossroads of innate and adaptive immunity. Annual Review of Cell and Developmental Biology 21, 511-527

Kaattari SL (1992): Fish B lymphocytes: Defining their form and function. In: Faisal M, Hetrick FM (eds.): Annual Review of Fish Diseases. Vol. 2. Pergamon, Tarrytown, NY, USA. 161–180

Kai L. (1993) Acquired immunity to infectious diseases in fish: implications for the interpretation of fish disease surveys. In:Fish: Ecotoxicology and Ecophysiology (ed. by T. Braunbeck, W. Hanke & H. Segner), Verlag Chemie, Weinheim, 183–196

Kai YH, Chi SC (2008) Efficacies of inactivated vaccines against betanodavirus in grouper larvae (Epinephelus coioides) by bath immunization. Vaccine 26, 1450–1457

Kai YH, Su HM, Tai KT, Chi SC (2010) Vaccination of grouper broodfish (Epinephelus tukula) reduces the risk of vertical transmission by nervous necrosis virus. Vaccine 28, 996–1001

Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, Kawashima S, Katayama T, Araki M, Hirakawa M (2006) From genomics to chemical

genomics: new developments in KEGG. Nucleic Acids Research 34, D354-D357

Kinkel M.D., Eames S.C., Philipson L.H., Prince V.E. (2010) Intraperitoneal injection into adult zebrafish. Journal of Visualized Experiments 42, doi:10.3791/2126

Koe CI, Chong SC (2011) Proteomics of buccal cavity mucus in female tilapia fish (Oreochromis spp.): A comparison between parental and non-parental fish. Plos ONE 6, e18555. doi: 10.1371/journal.pone.0018555

Kokoshis P.L., Williams D.L., Cook J.A. & Di Luzio N.R. (1978) Increased resistance to Staphylococcus aureus infection and enhancement in serum lysozyme activity by glucan. Science 199, 1340–1342

Köllner B, Wasserrab B, Kotterba G, Fischer U (2002) Evaluation of immune function of rainbow trout (Oncorhynchus mykiss)-how can environmental influences be detected? Toxicology Letters 131, 83-95

Kunlaya S., Vorrapon C., Wang H.C., Lo C.F. & Anchalee T. (2010) Proteomic analysis of differentially expressed proteins in Penaeus monodon hemocytes after Vibrio harveyi infection. Proteome Science 8, 39

Landolt ML. (1989) The relationship between diet and the immune response in fish. Aquaculture 79, 193-206

Lauren A.Z. & Hao S. (2007) Innate and adaptive immune responses to Listeria monocytogenes: a short overview. Microbes and Infection 9, 1208–1215

Lee K.K. (1995) Pathogenesis studies on Vibrio alginolyticus in the grouper, Epinephelus malabaricus, Bloch et Schneider. Microbial Pathogenesis 19, 39–48

Lee KK, Liu PC, Chuang WH (2002) Pathogenesis of gastroenteritis caused by Vibrio carchariae in cultured marine fish. Marine Biotechnology 4, 267–277

Lefkowitz RJ, Shenoy SK (2005) Transduction of receptor signals by beta-arrestins. Science. 308, 512-517

Leyens G., Donnay I. & Knoops B. (2003) Cloning of bovine peroxiredoxinse gene expression in bovine tissues and amino acid sequence comparison with rat, mouse and primate peroxiredoxins. Comparative Biochemistry and Physiology Part B 136, 943–955

Li J, Woo NYS (2003) Pathogenicity of vibrios in fish: an overview. Journal of Ocean University of China 2, 117–128

Li P, Ponnala L, Gandotra N, Wang L, Si Y, Tausta SL, Kebrom TH, Provart N, Patel R, Myers CR, Reidel EJ, Turgeon R, Liu P, Sun Q, Nelson T, Brutnell TP (2010) The developmental dynamics of the maize leaf transcriptome. Nature Genetics 42, 1060-1067

Li R.W. & Waldbieser G.C. (2006) Genomic organisation and expression of the natural killer cell enhancing factor (NKEF) gene in channel catfish, Ictalurus punctatus (Rafinesque). Fish and Shellfish Immunology 20, 72–82

Li SH, Zhang XJ, Sun Z, Li FH, Xiang JH (2013) Transcriptome analysis on Chinese shrimp Fenneropenaeus chinensis during WSSV acute infection. PlosONE 8(3): e58627. Doi:10.1371/journal.pone.0058627

Liao IC, Su HM, Chang EY (2001) Techniques in finfish larviculture in Taiwan. Aquaculture 200, 1–31

Lie O., Evensen O., Sorensen A. & Froysadal E. (1989) Study on lysozyme activity in some fish species. Diseases of Aquatic Organisms 6, 1–5

Lin CC, Lin JHY, Chen MS, Yang HL (2007) An oral nervous necrosis virus vaccine that induces protective immunity in larvae of grouper (Epinephelus coioides). Aquaculture 268, 265–273

Lin YH, Shiau SY (2005) Dietary L-ascorbic acid affects growth, nonspecific immune response and disease resistance in juvenile grouper Epinephelus malabaricus. Aquaculture 244, 215–221

Lionel BI. (2011) Inflammatory signaling in macrophages: Transitions from acute to tolerant and alternative activation states. European Journal of Immunology 41, 2477-2481

Litman G.W., Rast J.P., Shamblott M.J., Haire R.N., Hulst M., Roess W., Litman R.T., Hinds-Frey K.R., Zilch A. & Amemiya C.T. (1993) Phylogenetic diversification of immunoglobulin genes and the antibody repertoire. Molecular Biology and Evolution 10, 60–72

Livolsi A, Busuttil V, Imbert V, Abraham RT, Peyron JF. (2001) Tyrosine phosphorylation-dependent activation of NF-κB. Requirement for p56 LCK and ZAP-70 protein tyrosine kinases. European Journal of Biochemistry 268, 1508–1515

Love DR, Pichler FB, Dodd A, Copp BR, Greenwood DR (2004) Technology for high-throughput screens: the present and future using zebrafish. Current Opinion in Biotechnology15, 564–571

Lu MW, Ngou FH, Chao YM, Lai YS, Chen NY, Lee FY, Chiou PW (2012) Transcriptome characterization and gene expression of Epinephelus spp in endoplasmic reticulum stress-related pathway during betanodavirus infection in vitro. BMC genomics 13, 651-663

Luster AD. (2002) The role of chemokines in linking innate and adaptive immunity. Current Opinion in Immunology 14, 129-135

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Research 18, 1509-1517

McCarthy S.A., DePaola A., Cook D.W., Kaysner C.A. & Hill W.E. (1999) Evaluation of alkaline phosphatase- and digoxigenin-labelled probes for detection of the thermolabile hemolysin (tlh) gene of Vibrio parahaemolyticus. Letters in Applied Microbiology 28, 66–70

Meijer AH, Gabby Krens SF, Medina Rodriguez IA, He S, Bitter W, Ewa Snaar- Jagalska B, Spaink HP (2004) Expression analysis of the Toll-like receptor and TIR domain adaptor families of zebrafish. Molecular Immunology 40, 773–783

Melba GBR, Rohana PS, Richard AJ, Kazuo O, Supranee C, Robert A, Tan ZL and Shariff M (2005) Disease and health management in Asian aquaculture. Veterinary Parasitology 132, 249-272

Mesa G, Longobardi A, Sacco F, Marino G (2008) First release of hatchery juveniles of the dusky grouper Epinephelus marginatus (Lowe, 1834) (Serranidae: Teleostei) at artificial reefs in the Mediterranean: results from a pilot study. Scientia Marina 72, 743–756

Moore CA, Milano SK, Benovic JL (2007) Regulation of receptor trafficking by GRKs and arrestins. Annual Review of Physiology 69, 451-482

Mu Y, Ding F, Cui P, Ao J, Hu S, Chen X. (2010) Transcriptome and expression profiling analysis revealed changes of multiple signaling pathways involved in immunity in the large yellow croaker during *Aeromonas hydrophila* infection. BMC Genomics 11, 506. doi: 10.1186/1471-2164-11-506

Munday B.L. & Nakai T. (1997) Nodaviruses as pathogens in larval and juvenile marine finfish. World Journal of Microbiology and Biotechnology 13, 375–381

Na SY, Kang BY,Chung SW,Han SJ,MaX, TrinchieriG, Im SY (1999) Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NFkappaB. Journal of Biological Chemistry 274, 7674–7680

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 320, 1344-1349

Nagasawa K, Cruz-Lacierda ER (eds.) (2004) Diseases of cultured groupers. Southeast Asian Fisheries Development Center, Aquaculture Department, Iloilo, Philippines. 81 p

Neuberger M., Ehrenstein M., Rada C., Sale J., Batista F., Williams G. & Milstein C. (2000) Memory in the B-cell compartment: antibody affinity maturation. Philosophical Transactions of the Royal Society of London. Series B, Biological sciences 355, 357–360

Neumann NF, Stafford JL, Barreda D, Ainsworth AJ, Belosevic M (2001) Antimicrobial mechanisms of fish phagocytes and their role in host defense. Developmental and Comparative Immunology 25, 807-825

Noriea N.F. III, Johnson C.N., Griffitt K.J. & Grimes D.J. (2010) Distribution of type III secretion systems in Vibrio parahaemolyticus from the northern Gulf of Mexico. Journal of Applied Microbiology 109, 953–962

Onara D.F., Forlenza M., Gonzalez S.F., Rakus K.L., Pilarczyk A., Irnazarow I. & Wiegertjes G.F. (2008) Differential transcription of multiple forms of alpha-2-macroglobulin in carp (Cyprinus carpio) infected with parasites. Developmental and Comparative Immunology 32, 339–347

Or-Guil M., Wittenbrink N., Weiser A.A. & Schuchhardt J. (2007) Recirculation of germinal center B cells: a multilevel selection strategy for antibody maturation. Immunological Reviews 216, 130–141

Oshiumi H, Tsujita T, Shida K, Matsumoto M, Ikeo K, Seya T (2003) Prediction of the prototype of the human Toll-like receptor gene family from the pufferfish, Fugu rubripes, genome. Immunogenetics 54, 791–800

Pastoret PP, Griebel P, Bazin H, Govaerts A (1998) Handbook of Vertebrate Immunology. Academic Press, San Diego

Patrick J.B., Christine T., Michele D., Michele A., Marie-Andree A. & Bernard T. (1997) Both apolipoprotein E and A-I genes are present in a non-mammalian vertebrate and are highly expressed during embryonic development. Proceedings of the National Academic Sciences 94, 8622–8627

Paul R, Teresa F (2003) Management of environmental impacts of marine aquaculture in Europe. Aquaculture 226,139-163

Pereiro P, Balseiro P, Romero A, Dios S, Forn-Cuni G, Fuste B, Planas JV, Beltran S, Novoa B, Figueras A. (2012) High-throughput sequence analysis of turbot (*Scophthalmus maximus*) transcriptome using 454-pyrosequencing for the discovery of antiviral immune genes. PLoS One 7(5):e35369. doi: 10.1371/journal.pone.0035369

Pradipta RR, Bismita N and Surajit D (2012) Immune system and immune responses in fish and their role in comparative immunity study: A model for higher organisms. Immunology Letters 148, 23-33

Pradipta RR, Mrinal S, Hirak RD, Bismita N, Surajit D (2014) Toll-like receptors (TLRs) in aquatic animals: Signaling pathways, expressions and immune responses. Immunology Letters 158, 14-24

Prager E.M. & Jollès P. (1996) Animal lysozymes c and g: an overview. In: Lysozymes: Model Enzymes in Biochemistry and Biology (ed. by P. Jollès), Birkhuser Verlag, Basel, 9–31

Press CM and Evensen O. (1999) The morphology of the immune system in teleost fishes. Fish and Shellfish Immunology 9, 309-318

Punitha SMJ, Babu MM, Sivaram V, Shankar VS, Dhas SA, Mahesh TC, Immanuel G, Citarasu T (2008) Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper Epinephelus tauvina juvenile against Vibrio harveyi infection. Aquaculture International 16, 511–523

Qin QW, Chang SF, Ngoh-Lim GH, Gibson-Kueh S, Shi C, Lam TJ (2003) Characterization of a novel ranavirus isolated from grouper Epinephelus tauvina. Diseases of Aquatic Organisms 53, 1–9

Qin QW, Lam TJ, Sin YM, Shen H, Chang SF, Ngoh GH, Chen CL (2001) Electron microscopic observations of a marine fish iridovirus isolated from brown-spotted grouper, Epinephelus tauvina. Journal of Virological Methods 98, 17–24

Qin QW, Shi C, Gin KY, Lam TJ (2002) Antigenic characterization of a marine fish iridovirus from grouper, Epinephelus spp. Journal of Virological Methods 106, 89–96

Qin QW, Wu TH, Jia TL, Hegde A, Zhang RQ (2006) Development and characterization of a new tropical marine fish cell line from grouper,

Epinephelus coioides susceptible to iridovirus and nodavirus. Journal of Virological Methods 131, 58–64

Qin QW, Wu ZH, Pan JP (2000) Disease resistance and humoral immunomodulatory effect of vitamin C on grouper, Epinephelus awoara. Chinese Journal of Oceanology and Limnology 18, 247–252

Ramasamy H, Chellam B, Heo MS (2010) Molecular studies, disease status and prophylactic measures in grouper aquaculture: Economic importance, diseases and immunology. Aquaculture 309, 1-14

Ramasamy H, Kim DH, Hong SH, Pitchaimuthu M, Chellam B, Heo MS (2012) Non-specific immune response and disease resistance induced by Siegesbeckia glabrescens against Vibrio parahaemolyticus in Epinephelus bruneus. Fish and Shellfish Immunology 33, 359-364

Rebl A, Goldammer T, Seyfert HM (2010) Toll-like receptor signaling in bony fish. Veterinary Immunology and Immunopathology 134, 139–150

Rimmer MA (1998) Grouper and snapper aquaculture in Taiwan. Austasia Aquaculture 12, 3–7

Rimmer MA, McBride S, and Williams KC (2004) Advances in grouper aquaculture. Australian Centre for International Agricultural Research. Monograph 110. ISBN 1 86320 438 5 (printed)

Rimmer MA, Williams KC, Phillips MJ (2000) Proceedings of the Grouper Aquaculture Workshop held in Bangkok, Thailand, 7–8 April 1998, Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand

Rombout JHWM, Taverne N, van de Kamp M, Taverne-Thiele AJ (1993) Differences in mucus and serum immunoglobulin of carp (Cyprinus carpio L.). Developmental and Comparative Immunology 17, 309-317

Sadovy YJ, Donaldson TJ, Graham TR, McGilvray F, Muldoon GJ, Phillips MJ, Rimmer MA, Smith A, Yeeting B (2003) While Stocks Last: The Live Reef Food Fish Trade, Asian Development Bank, Manila, Philippines

Saeed MO (1995) Association of Vibrio harveyi with mortalities in cultured marine fish in Kuwait. Aquaculture 136, 21–29

Samitas K., Vittorakis S., Chorianopoulos D., Oikonomidou E. & Gaga M. (2007) Immunological mechanisms in the lung. Pneumonia 20, 274–278

Sammalkorpi K., Valtonen V., Kerttula Y., Nikkila E. & Taskinen M.R. (1988) Changes in serum lipoprotein pattern induced by acute infections. Metabolism 37, 859–865

Sankar GP., Saravanan J., Krishnamurthy P., Chandrakala N. & Rajendran K. (2012) Isolation and identification of Vibrio spp. in diseased Channa punctatus from aquaculture fish farm. Indian Journal of Geo-Marine Sciences 41, 159–163

Santha S., Puthenkandathil S.D., Bini F. & Ammanamveetil A.M.H. (2012) Prevalence and distribution of Vibrio parahaemolyticus in finfish from Cochin (South India). Veterinaria Italiana 48, 269–281

Saurabh S. & Sahoo P.K. (2008) Lysozyme: an important defence molecule of fish innate immune system. Aquaculture Research 39, 223–239

Sauri H., Butterfield L., Kim A. & Shau H.Y. (1995) Antioxidant function of recombinant human natural killer enhancing factor. Biochemical and Biophysical Research Communications 208, 964–969

Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. (2011) The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochimica et Biophysica Acta 1813, 878-888

Secombes C.J. (1996). The nonspecific immune system: cellular defenses. In: The Fish Immune System: Organism, Pathogen and Environment (ed. by G. Iwama & T. Nakanishi), Academic Press, San Diego, CA, USA. 63–103

Secombes C.J. & Chappell L.H. (1996) Fish immune responses to experimental and natural infection with helminth parasites. Annual Review of Fish Diseases 6, 167–177

Seng LT (1998) Grouper Culture. In: de Silva, S.S. (Ed.), Tropical mariculture. Academic Press, London, 423–448

Shariff M (1995) Fish health: an odyssey through the Asia-Pacific region. Syarahan inaugural. Univ. Pertanian Malaysia, Serdang, Malaysia, 25 pp

Shau H.Y., Gupta R.K. & Golub S.H. (1993) Identification of a natural killer enhancing factor (NKEF) from human erythroid cells. Cellular Immunology 147, 1–11

Shona KW (2007) The innate immune response of finfish – A review of current knowledge. Fish and Shellfish Immunology 23, 1127-1151

Shruti C. & Soumya H. (2012) Vibrio related diseases in aquaculture and development of rapid and accurate identification methods. Journal of Marine Science Research and Development S1, 002

Sinyakov MS, Dror M, Zhevelev HM, Margel S, Avtalion RR (2002) Natural antibodies and their significance in active immunization and protection against a defined pathogen in fish. Vaccine 20, 3668-3674

Sitja-Bobadilla A., Redondo M.J., Bermudez R., Palenzuela O., Ferreiro I., Riaza A., Quiroga I., Nieto J.M. & Alvarez-Pellitero P. (2006) Innate and adaptive immune responses of turbot, Scophthalmus maximus (L.), following experimental infection with Enteromyxum scophthalmi (Myxosporea: Myxozoa). Fish and Shellfish Immunology 21, 485–500

Sivaram V, Babu MM, Immanuvel G, Murugadass S, Citarasu T, Marian MP (2004) Grouth and immune response of juvenile greasy (Epinephelus tauvina) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture 237, 9–20

Sugama K (2003) Indonesia focuses on groupers. Asian Aquaculture Magazine September–October 2003, 14–15

Sun Y, Yang H, Ling Z, Chang J, Ye J (2009) Gut microbiota of fast and slow growing grouper Epinephelus coioides. African Journal of Microbiology Research 3, 713–720

Sunyer JO, Tort L (1995) Natural hemolytic and bactericidal activities of sea bream Sparus aurata are effected by the alternative complement pathway. Veterinary Immunology and Immunopathology 45, 333-345

Sunyer JO, Tort L, Lambris JD (1997) Diversity of the third form of complement, C3, in fish: functional characterization of five forms of C3 in the diploid fish Sparus aurata. Biochemical Journal 326, 877-881

Swoboda I., Bugajska-Schretter A., Verdino P., Keller W., Sperr W.R., Valent P., Valenta R. & Spitzauer S. (2002) Recombinant carp parvalbumin, the major cross-reactivity fish allergen: a tool for diagnosis and therapy of fish allergy. The Journal of Immunology 168, 4576–4584

Takano T, Hwang SD, Kondo H, Hirono I, Aoki T, Sano M (2010) Evidence of molecular Toll-like receptor mechanisms in teleosts. Fish Pathology 45, 1–16

Tania C.S., Lidiane Z.G., Evilin N.K., Anderson D.R., Katia C., Noemia M.O., Monica L.F.&Carla L. (2011) Nattectin a fish C-type lectin drives Th1 responses in vivo: licenses macrophages to differentiate into cells exhibiting typical DC function. International Immunopharmacology 11, 1546–1556

Tatusov RL, Galperin MY, Natale DA, Koonin EV. (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Research 28, 33-36

Tcherynchev B, Cabilly S, Wilchek M (1997) The epitopes for natural polyreactive antibodies are rich in proline. Proceedings of the Natural Academy of Sciences of the United States of America 94, 6335-6336

Tekwani BL, Shukla OP and Ghatak S. (1988). Altered drug metabolism in parasitic diseases. Parasitology Today, 4, 4-10

Thushara WM, Ingrid EL, Nicholas PD, Joann M and Aaron MR (2013) A transcriptomic approach to elucidate the physiological significance of human cytochrome P450 2S1 in bronchial epithelial cells. BMC Genomics 14, 833-845

Uribe C, Folch H, Enriquez R, Moran G (2011) Innate and adaptive immunity in teleost fish: a review. Veterinarni Medicina 56, 486-503

Vera JC, Wheat CW, Fescemyer HW, Frilander MJ, Crawford DL, Hanski I, Marden JH (2008) Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing. Molecular Ecology 17, 1636-1647

Weaver DE (2006) Design and operations of fine media fluidized bed biofilters for meeting oligotrophic water requirements. Aquacultural Engineering 34, 303-310

Wei J, Xu D, Zhou J, Cui H, Yan Y, Ouyang Z, Gong J, Huang Y, Huang X, Qin Q (2010) Molecular cloning, characterization and expression analysis of a C-type lectin (Ec-CTL) in orange-spotted grouper, Epinephelus coioides. Fish and Shellfish Immunology 28, 178–186

Wen CM, Lee CW, Wang CS, Cheng YH, Huang HY (2008) Development of two cell lines from Epinephelus coioides brain tissue for characterization of betanodavirus and megalocytivirus infectivity and propagation. Aquaculture 278, 14–21

Werts C, Tapping RI, Mathison JC (2001) Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nature Immunology 2, 346–352

Wilson M, Bengten E, Miller NW, Clem LW, Du Pasquier L, Warr GW (1997) A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. Proceedings of the National Academy of Sciences USA 94, 4593–4597

Wong SY, Leong TS (1987) Current fish disease problems in Malaysia, pp. 12–21. In: Arthur, J.R. (Ed.). Fish Quarantine and Fish Diseases in South and Southeast Asia: 1986 Update. Asian Fisheries Society Special Publication No. 1

Wong S.Y. & Leong T.S. (1990) A comparative study of Vibrio infections in healthy and diseased marine finfishes cultured in floating cages near Penang, Malaysia. Asian Fisheries Science 3, 353–359

Wu Y., Wang S. & Peng X. (2004) Serum acute phase response (APR)-related proteome of loach to trauma. Fish and Shellfish Immunology 16, 381–389

Xia JH, Yue GH (2010) Identification and analysis of immune-related transcriptome in Asian seabass Lates calcarifer. BMC Genomics 11, 356-368

Xiong XP, Dong CF, Xu XP, Weng SP, Liu ZY, He JG (2011) Proteomic analysis of zebrafish (Danio rerio) infected with infectious spleen and kidney necrosis virus. Developmental and Comparative Immunology 35, 431-440

Yambot AV, Song YL (2006) Immunization of grouper, Epinephelus coioides, confers protection against a protozoan parasite, Cryptocaryon irritans. Aquaculture 260, 1–9

Yambot AV, Song YL, Sung HH (2003) Characterization of Cryptocaryon irritans, a parasite isolated from marine fishes in Taiwan. Diseases of Aquatic Organisms 54, 147–156

Yin Z.X., He J.G., Deng W.X. & Chan S.M. (2003) Molecular cloning, expression of orange spotted grouper goose-type lysozyme cDNA, and lytic activity of its recombinant protein. Diseases of Aquatic Organisms 55, 117–123

Yin ZX, He W, Chen WJ, Yan JH, Yang JN, Chan SM, He JG (2006) Cloning, expression and antimicrobial activity of an antimicrobial peptide, epinecidin-1, from the orange-spotted grouper, Epinephelus coioides. Aquaculture 253, 204–211

Yniv P (2011) Toll-like receptors in bony fish: From genomic to function. Developmental and Comparative Immunology 35, 1263-1272

Yoshinaga T, Nakazoe J (1997) Acquired protection and production of immobilization antibody against Cryptocaryon irritans (Ciliophora, Hymenostomatida) in mummichog (Fundulus heteroclitus). Fish Pathology 32, 229–230

Zarkadis IK, Mastellos D, Lambris JD (2001) Phylogenetic aspects of the complement system. Developmental and Comparative Immunology 25, 745-762

Zeng DG, Chen XL, Xie DX, Zhao YZ, Yang CL, Li YM, Ma N, Peng M, Yang Q, Liao ZP, Wang H, Chen XH (2013) Transcriptome analysis of pacific white shrimp (Litopenaeus vannamei) hepatopancreas in response to taura syndrome virus (TSV) experimental infection. PlosONE 8(2): e57515. Doi:10.1371/journal.pone.0057515

Zhang H., Evenhuis J.P., Thorgaard G.H. & Ristow S.S. (2001) Cloning, characterization and genomic structure of the natural killer cell enhancement factor (NKEF)-like gene from homozygous clones of rainbow trout (Oncorhynchus mykiss). Developmental and Comparative Immunology 25, 25–35

Zhang YA, Salinas I, Li J, Parra D, Bjork S, Xu Z, Lapatra SE, Bartholomew J, Sunyer JO (2010) IgT, a primitive immunoglobulin class specialized in mucosal immunity. Nature Immunology 11, 827–835

Zimmerman A.M., Evenhuis J.P., Thorgaard G.H. & Ristow S.S. (2004) A single major chromosomal region controls natural killer cell-like activity in rainbow trout. Immunogenetics 55, 825–835

Zoltán H, Anna Z, Vilmos CÁ, Anita O, Péter R, Mátyás M, Herman PS, Annemarie HM (2009) Deep sequencing of the zebrafish transcriptome response to mycobacterium infection. Molecular Immunology 46, 2918-2930

Zuo X. & Woo P.T.K. (1997) Natural anti-proteases in rainbow trout, Oncorhynchus mykiss and brook charr, Salvelinus fontinalis and the in vitro neutralization of fish alpha 2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate, Cryptobia salmositica. Parasitology 114, 375