



**UNIVERSITI PUTRA MALAYSIA**

***DEVELOPMENT AND EVALUATION OF RECOMBINANT VECTOR CELLS  
CARRYING CELL WALL SURFACE ANCHOR FAMILY PROTEINS AS A  
VACCINE AGAINST STREPTOCOCCOSIS IN RED HYBRID TILAPIA  
(OREOCHROMIS spp)***

**NUR NAZIFAH BINTI MANSOR**

**FPV 2013 8**



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**DOCTOR OF PHILOSOPHY  
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**DEVELOPMENT AND EVALUATION OF RECOMBINANT VECTOR CELLS  
CARRYING CELL WALL SURFACE ANCHOR FAMILY PROTEINS AS A VACCINE  
AGAINST STREPTOCOCCOSIS IN RED HYBRID TILAPIA (*Oreochromis*  
*spp*)**

**NUR NAZIFAH MANSOR**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Philosophy**

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

**DEVELOPMENT AND EVALUATION OF RECOMBINANT VECTOR CELLS  
CARRYING CELL WALL SURFACE ANCHOR FAMILY PROTEINS AS A  
VACCINE AGAINST STREPTOCOCCOSIS IN RED HYBRID TILAPIA  
(*Oreochromis spp*)**

By

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**July 2013**

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**Faculty : Veterinary Medicine**

Tilapia is one of the most common cultured fish in many countries. However, productions of tilapia might decrease due to various diseases, including streptococcosis that can kill 100% of the fish. In Malaysia, outbreaks of streptococcosis are frequently observed during the dry months, particularly between April and August when the water temperature is high. Streptococcosis in fish is caused by either *Streptococcus agalactiae* or *S. iniae*. Although vaccination is practiced to control streptococcosis, the protection remains unclear. Thus, this project attempted to develop and evaluate recombinant cell for vaccine preparation to improve vaccine efficacy against streptococcosis. The hypothesis of this study are major outer surface protein of *Streptococcus agalactiae* is suitable as vaccine candidate and can be cloned and expressed in

the *Escherichia coli* prokaryotic system to produce the recombinant vaccine. The newly developed feed based recombinant vaccine can elicit certain level of systemic and mucosal antibody and gave protection against Streptococcosis in red hybrid tilapia.

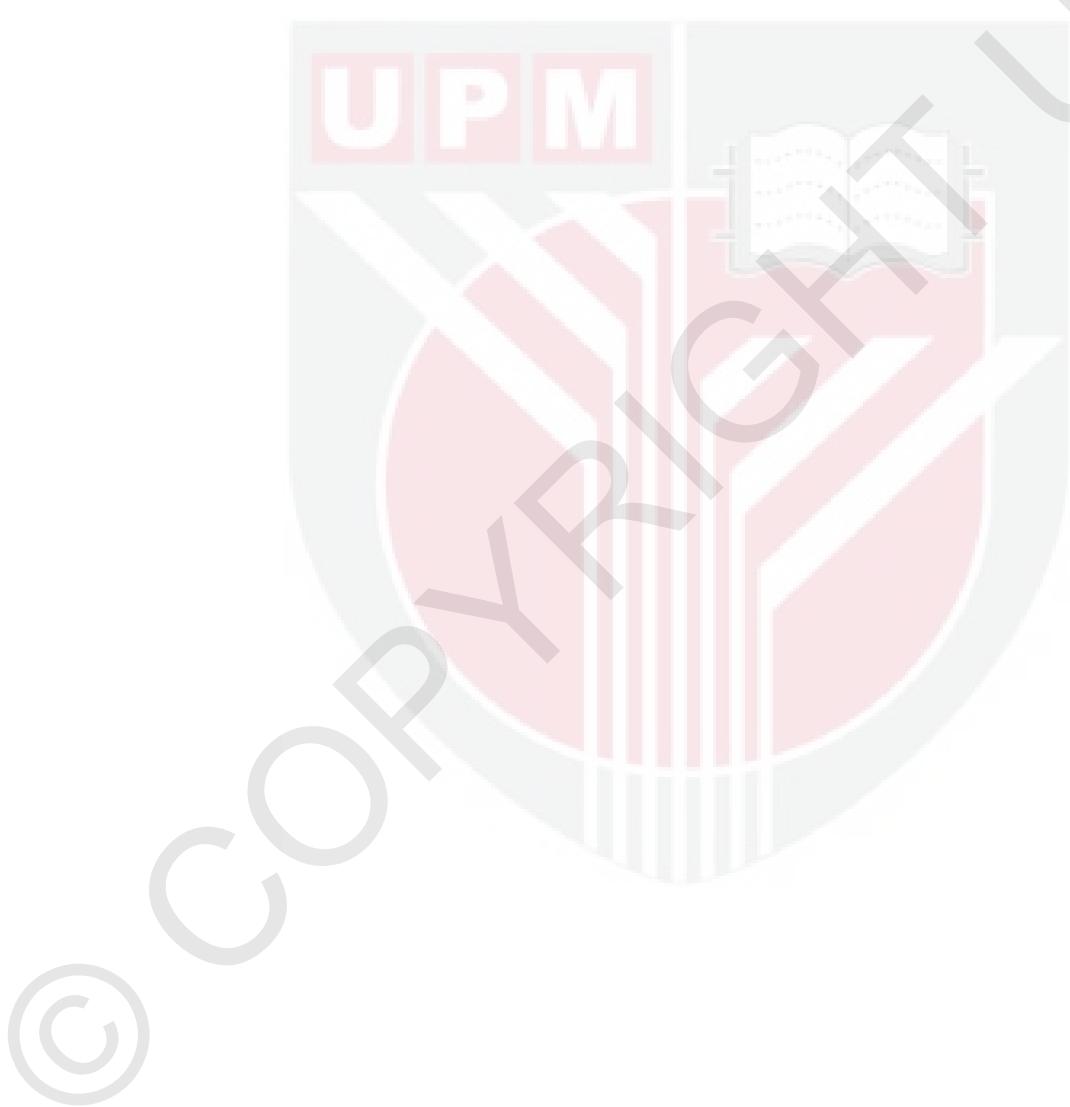
The outer surface proteins (OSPs) of *S. agalactiae* isolated earlier from fish that died of streptococcosis were extracted, purified and characterized. The 48kDa protein band was found to be the most antigenic following SDS-PAGE and Western immunoblotting methods. Therefore, the 48kDa protein band was selected for further studies as a vaccine candidate.

The 48kDa band was processed for N-terminal sequencing, and the results revealed that the protein was the cell wall surface anchor family protein of the outer surface protein (OSP) of *S. agalactiae*, which was encoded by a gene of approximately 1263bp in size. The DNA gene was amplified by the polymerase chain reaction (PCR) method, purified and cloned in pET-32 Ek/Lic vector. The successful clones were then transformed into Novablue *Escherichia coli* strain before the positive clones were screened for the end product of 1335bp; the vector contributed 72bp. Plasmid extraction was performed prior to the expression of the cell wall surface anchor family protein in the BL21 (DE3) *Escherichia coli*. Overnight Express™ Autoinduction system 1 (Novagen, USA) was used in expression. Successful protein expression was analysed by SDS-PAGE and western immunoblotting and was found to be approximately 65.8kDa, consisted of the 48kDa cell wall surface anchor family protein and the 17.8kDa of tagged protein.

The successful expression of the protein of interest paved the way for preparation of crude recombinant vaccine against streptococcosis. Next was the study on humoral and mucosal immune responses by red hybrid tilapia following exposure to the inactivated recombinant cell vaccine. The newly developed recombinant vaccine was incorporated homogenously into fish pellet at a concentration of  $1 \times 10^6$  CFU/g as feed-based vaccine against streptococcosis. To vaccinate, red hybrid tilapia were given the feed-based recombinant vaccine orally before a booster dose was given 2 weeks later. The results showed that the newly develop recombinant vaccine elicited high levels of systemic and mucosal immunity from week-5 until the end of the 12-week study period.

Lastly, the efficacy of the newly developed feed-based recombinant vaccine against Streptococcosis in red hybrid tilapia (*Oreochromis* spp.) was tested. Following initial vaccination and booster dose at week 2, the fish were challenged intraperitoneal on week 6 with an inoculum containing  $2.27 \times 10^9$  CFU/mL of live *S. agalactiae*. This was followed by heat stress at 31°C throughout the entire 3-week challenge period, which ended on week 9. The newly develop feed-based recombinant vaccine stimulated both mucosal and systemic immunity when the immunoglobulin levels were higher, the size of GALT was bigger and the number of lymphocytes was greater. The stimulations, however, were able to provide only 70% protection. Nevertheless, the efficacy of the newly develop recombinant vaccine was significantly better than the whole cell vaccine.

In conclusion, the 70% protection provided by the newly developed recombinant vaccine was considered as moderate. Therefore, vaccine enhancement should be carried out in the future to produce an excellent protection against streptococcosis. Adding oil adjuvant to the vaccine can be considered as one of the approaches to further enhance the efficacy of the recombinant vaccine.



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

**PEMBANGUNAN DAN PENILAIAN SEL REKOMBINAN VEKTOR YANG  
MEMBAWA "PROTEIN FAMILI DINDING SEL PERMUKAAN SAUH" SEBAGAI  
VAKSIN TERHADAP PENYAKIT STREPTOCOCCOSIS DALAM IKAN TILAPIA  
MERAH HIBRID (*OREOCHROMIS* spp)**

Oleh

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Tilapia adalah sejenis ikan yang biasa diternak di kebanyakan negara. Walau bagaimanapun, pengeluaran ikan tilapia mungkin berkurangan disebabkan oleh pelbagai penyakit termasuk penyakit Streptococcosis yang boleh menyebabkan 100% kematian. Di Malaysia, wabak penyakit ini berlaku semasa musim kemarau antara bulan April dan Ogos setiap tahun iaitu apabila suhu air adalah tinggi. Penyakit Streptococcosis pada ikan adalah disebabkan oleh *S. agalactiae* atau *S. iniae*. Walaupun kaedah pevaksinan telah digunakan untuk mengawal penyakit ini, hasilnya masih tidak jelas. Oleh itu, projek ini telah dijalankan bagi membangunkan dan menilai sel rekombinan yang akan digunakan dalam penyediaan vaksin untuk meningkatkan keberkesanan vaksin terhadap

penyakit Streptococcosis. Hipotesis bagi kajian ini adalah protin permukaan luar dari *Streptococcus agalactiae* yang major sesuai dijadikan sebagai calon vaksin dan boleh diklon dan diekspresikan dalam system prokaryotik *Escherichia coli* bagi penghasilan vaksin rekombinan. Vaksin rekombinan berasaskan makanan yang baru dibangunkan ini dipercayai boleh menghasilkan tahap antibody sistemik dan mukosal tertentu dan memberi perlindungan kepada ikan tilapia merah hibrid terhadap penyakit Streptococcosis.

Protein permukaan luar (OSPs) dari *S. agalactiae* yang dipencil daripada ikan Tilapia yang telah mati disebabkan oleh penyakit Streptococcosis telah diekstrak, disucikan dan dicirikan. Protein 48 kDa yang dijalurkan adalah protein yang paling antigenik yang ditentusahkan menggunakan kaedah analisis "SDS PAGE" dan Pemplotan Western. Oleh itu, jalur protein 48 kDa telah dipilih untuk dikaji dengan lebih terperinci dan dijadikan sebagai calon vaksin.

Jaluran protein 48 kDa telah diproses untuk penujujukan Terminal-N dan keputusannya menunjukkan ianya adalah " Protein keluarga dinding sel permukaan sauh" dari protein permukaan luar (OSPs) yang terdapat pada *S. agalactiae*. Protein ini mengkodkan jujukan gen DNA bersaiz 1263 bp. Gen DNA tersebut telah diamplifikasi menggunakan kaedah tindakbalas rantai polimerase (PCR), disucikan dan diklonkan di dalam vektor pET-32 Ek/LIC. Klon yang berjaya dihasilkan telah dimasukkan kedalam bakteria Novablue *E. coli*. Klon positif telah diskrin menggunakan kaedah PCR dan menghasilkan jujukan bersaiz 1335 bp; 72 bp disumbangkan oleh vektor. Kemudian, pengekstrakan plasmid telah dijalankan supaya dapat digunakan dalam proses

ekspresi protein bagi “protin keluarga dinding sel permukaan sauh” di dalam bakteria BL21 (DE3) *E. coli*. Pengaruan ekspresi protin dijalankan dengan menggunakan kit “ Overnight Express™ Autoinduction System 1” (Novagen, USA). Protin yang telah berjaya diekspreskan, dianalisa dengan menggunakan kaedah analisis “SDS PAGE” dan Pemplotan Western. Protin tersebut adalah bersaiz 65.8 kDa iaitu berpadanan dengan saiz “Protin keluarga dinding sel permukaan sauh”, 48 kDa dan protin.Tag bersaiz 17.8 kDa.

Ekspesi protin telah berjaya dihasilkan dan membuka jalan dalam penyediaan vaksin rekombinan mentah yang sesuai digunakan bagi mengawal penyakit Streptococcosis. Seterusnya adalah kajian yang dijalankan bagi menentukan tindakbalas imun humoral dan mukosa terhadap sel rekombinan vaksin yang telah dilemahkan yang digunakan pada ikan Tilapia merah. Rekombinan vaksin yang baru dibangunkan ini digaul secara rata ke dalam pellet makanan ikan berkepekatan  $1 \times 10^6$  CFU/g menjadikannya vaksin rekombinan berasaskan makan bagi mengawal penyakit Streptococcosis. Pada pemvaksinan, ikan Tilapia merah telah diberikan vaksin rekombinan berasaskan makanan secara oral dan kemudiannya dos peningkatan diberikan dua minggu kemudian. Keputusan menunjukkan bahawa rekombinan vaksin yang baru dibangunkan berjaya menghasilkan kadar tindakbalas imun humoral dan mukosa yang tinggi dari minggu ke lima sehingga ke akhir eksperimen iaitu minggu ke dua belas.

Keberkesanan rekombinan vaksin berasaskan makanan yang baru dibangunkan terhadap Tilapia merah hibrid (*Oreochromis sp.*) diuji. Setelah diberikan

pemvaksinan awal dan dos peningkatan dua minggu kemudiannya, ikan Tilapia merah telah diuji secara suntikan intraperitonium pada minggu keenam menggunakan inokulum mengandungi  $2.27 \times 10^9$  CFU/mL *S. agalactiae* hidup. Ikan juga diberikan tekanan dengan kenaikan suhu air kepada 31°C bermula minggu keenam hingga minggu kesembilan; iaitu minggu terakhir eksperimen. Rekombinan vaksin berdasarkan makanan yang baru dibangunkan merangsang kedua-dua imun mukosa dan sistemik. Ketika tahap immunoglobulin adalah tinggi, saiz “sel limfoid berkaitan usus” (GALT) adalah besar, dan bilangan limfosid juga adalah tinggi. Walau bagaimanapun, dengan stimulasi tinggi yang dihasilkan, vaksin rekombinan berdasarkan makanan yang baru dibangunkan ini mampu memberi 70% perlindungan. Namun, keberkesanan vaksin rekombinan berdasarkan makanan yang baru dibangunkan ini secara ketaranya memberikan perlindungan yang lebih baik berbanding vaksin keseluruhan sel.

Kesimpulannya, 70% perlindungan yang diberikan oleh vaksin rekombinan berdasarkan makanan yang baru dibangunkan diklasifikasikan sebagai sederhana. Oleh itu, vaksin ini perlulah ditambahbaik di masa hadapan bagi menghasilkan perlindungan yang menyeluruh terhadap penyakit Streptococciosis. Penambah minyak Adjuvant terhadap vaksin tersebut boleh dijadikan sebagai salah satu langkah penambahbaikkan bagi meningkatkan lagi keberkesanan vaksin rekombinan berdasarkan makanan ini.

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I certify that a Thesis Examination Committee has met on 17 July 2013 to conduct the final examination of Nur Nazifah binti Mansor on her thesis entitled "Development And Evaluation of Recombinant Vector Cells Carrying Cell Wall Surface Anchor Family Proteins as a Vaccine Against Streptococcosis in Red Hybrid Tilapia (Oreochrimis Spp)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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## **DECLARATION**

I hereby declare that the thesis is based on my original work accept for quotation and citation, which have been duly acknowledged. I also declare that it has not been previously or concurrent submitted for any other degree at Universiti Putra Malaysia or other institutions.



**NUR NAZIFAH MANSOR**

Date: 17 July 2013

## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	x
<b>APPROVAL</b>	xiii
<b>DECLARATION</b>	xv
<b>LIST OF TABLES</b>	xxi
<b>LIST OF FIGURES</b>	xxii
<b>LIST OF ABBREVIATIONS</b>	xxxvi
 <b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	1
 <b>2 LITERATURE REVIEW</b>	5
2.1 Streptococcosis	5
2.2 <i>Streptococcus agalactiae</i>	7
2.2.1 Cell Morphology	7
2.3 Cell Wall of Gram Positive Bacteria	7
2.3.1 Capsule	9
2.3.2 Peptidoglycan	10
2.3.3 The Cytoplasmic membrane	10
2.4 Potential immunogenic agents of <i>Streptococcus Agalactiae's</i> cell wall	12
2.4.1 Outer Surface Protein (OSP)	12
2.4.2 Cell Wall Surface Anchor Family Protein	13
2.4.3 Phosphoglucomutase	15
2.4.4 Capsule	15
2.4.5 Rib Protein	16
2.4.6 C <sup>α</sup> -Protein	17
2.4.7 R5 Protein	18
2.5 Molecular Approaches to Vaccine Development	19
2.5.1 Type of Vaccine	19
2.5.2 Route of Vaccination in Aquaculture	22
2.6 Cloning and Protein Expression	25
2.6.1 Definition of Cloning and Protein Expression	25
2.6.2 Plasmid as the Expression Vector	27
2.6.3 <i>Escherichia coli</i> as Host Strain	28
2.6.4 Fusion Protein Technology	29

<b>3</b>	<b>CHARACTERIZATION OF THE OUTER SURFACE PROTEINS OF <i>STREPTOCOCCUS AGALACTIAE</i></b>	<b>31</b>
3.1	Introduction	31
3.2	Materials and Methods	36
3.2.1	Bacterial Strains	36
3.2.2	Preparation of Crude Whole Cells Protein of <i>S. agalactiae</i>	36
3.2.3	Preparation of Outer Surface Protein of <i>S. agalactiae</i>	37
3.2.4	Preparation of Rabbit Hyper-immune Serum Against The Crude Whole Cells Protein of <i>S. agalactiae</i>	38
3.2.5	Protein Separation by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Technique	39
3.2.6	Western Immunoblot	40
3.2.7	Gel and Membrane Analysis	42
3.3	Result	42
3.3.1	SDS PAGE	42
3.3.2	Antigenicity of the Outer Surface Protein of <i>S. agalactiae</i>	43
3.4	Discussion	48
<b>4</b>	<b>DEVELOPMENT OF A RECOMBINANT PLASMID CONTAINING THE CELL WALL SURFACE ANCHOR FAMILY PROTEIN-ENCODED GENE OF <i>STREPTOCOCCUS AGALACTIAE</i></b>	<b>50</b>
4.1	Introduction	50
4.2	Materials and Methods	53
4.2.1	Protein Sequence Analysis	53
4.2.2	Bacterial Culture Conditions	53
4.2.3	DNA Extraction	54
4.2.4	Polymerase Chain Reaction (PCR)	55
4.2.4.1	Primers	55
4.2.4.2	Gene Amplification by PCR	56
4.2.5	Detection of PCR Product	58
4.2.6	Gel Extraction and Purification of PCR Products	59
4.2.7	Construction of Recombinant Plasmid	60
4.2.7.1	Vector	60
4.2.7.2	T4 DNA Polymerase Treatment of Target Insert	60
4.2.7.3	Ligation of the Vector and Ek/Lic Insert	61
4.2.7.4	Transformation of Ligation Mixtures Into Competent <i>Escherichia coli</i>	62

4.2.7.5	Colony Screening	63
4.2.7.6	Plasmid Extraction	66
4.2.7.7	Analysis of Positive Recombinants by PCR	67
4.2.8	DNA and Protein Analysis	69
4.3	Results	70
4.3.1	Protein Profile of The 48kDa of Outer Surface Protein of <i>Streptococcus agalactiae</i>	70
4.3.2	Amplification of The Cell Wall Surface Anchor Family Gene from <i>Streptococcus agalactiae</i> Genome	70
4.3.3	Cloning of Cell Wall Surface Anchor Family Gene in <i>E. coli</i> cells	71
4.3.4	DNA Sequence Analysis of the Recombinant Plasmid	71
4.3.5	DNA Sequence Analysis of the Cell Wall Surface Anchor Famili Gene	71
4.4	Discussion	76
<b>5</b>	<b>EXPRESSION OF THE SURFACE ANCHOR FAMILY PROTEIN OF <i>STREPTOCOCCUS AGALACTIAE</i> IN <i>ESCHERICHIA COLI</i></b>	<b>80</b>
5.1	Introduction	80
5.2	Materials and Methods	83
5.2.1	Preparation of Rabbit Hyper-immune Serum Against The Cell Wall Surface Anchor Famili Protein of <i>S. agalactiae</i>	83
5.2.2	Transformation of Recombinant Plasmid into Expression Host	83
5.2.3	Isopropyl-beta-D-thiogalactopyranoside (IPTG) Auto-Induction	84
5.2.4	Protein Extraction	85
5.2.5	Recombinant Protein Analysis by SDS PAGE	86
5.2.6	Analysis of the Expressed Protein by Western Immunoblotting	86
5.3	Results	88
5.3.1	Transformation	88
5.3.2	Expression of pET-32Ek/LIC-Cell Wall Surface Anchor Family	90
5.4	Discussion	94

<b>6</b>	<b>SYSTEMIC AND MUCOSAL IMMUNITY RESPONSES BY OREOCHROMIS SPP. FOLLOWING VACCINATION WITH FEED-BASED RECOMBINANT VACCINE ENCODING THE CELL WALL SURFACE ANCHOR FAMILY PROTEIN OF <i>STREPTOCOCCUS AGALACTIAE</i></b>	100
6.1	Introduction	100
6.2	Material and Methods	103
6.2.1	Fish	103
6.2.2	Preparation of Inactivated Recombinant Cells	103
6.2.3	Preparation of Feed-Based Recombinant Vaccine	104
6.2.4	Experimental Procedure	105
6.2.5	Sampling Protocol for Serum, Mucus and Gut Lavage Fluid	108
6.2.6	Histopathology	109
6.2.7	Enzyme-Linked Immunosorbent Assay (ELISA)	110
6.2.8	Statistical Analysis	112
6.3	Result	112
6.3.1	Responses by Systemic Immunity	112
6.3.1.1	Serum Antibody Response	112
6.3.2	Responses by Mucosal Immunity	115
6.3.2.1	Antibody Level in The Mucus	115
6.3.2.2	Antibody Level in The Gut Lavage Fluid	117
6.3.2.3	Histological Analysis	119
6.3.2.3.1	Number of Lymphoid Cells	119
6.3.2.3.2	Size of GALT	120
6.3.2.3.3	Correlation Between Mucosal Antibody Levels and GALT	120
6.4	Discussion	127
<b>7</b>	<b>EFFICACY OF AN INACTIVATED RECOMBINANT VACCINE ENCODING THE CELL WALL SURFACE ANCHOR FAMILY PROTEIN OF <i>S. AGALACTIAE</i> AGAINST STREPTOCOCCOSIS IN <i>OREOCHROMIS SPP</i></b>	130
7.1	Introduction	130
7.2	Material and Methods	132
7.2.1	Fish	132
7.2.2	Preparation of Inactivated Recombinant Vaccine	132
7.2.3	Preparation of Feed-Based Recombinant Vaccine	132
7.2.4	Preparation of Live Bacterial Inoculums for Challenge	132

7.2.5	Experimental Procedure	133
7.2.6	Collection of Serum, Mucus and Gut Lavage	134
7.2.7	Histopathology	134
7.2.8	Enzyme-Linked Immunosorbent Assay (ELISA)	135
7.2.9	Bacterial Isolation, Gram Stain and PCR	137
7.2.10	Statistical Analysis	137
7.3	Results	138
7.3.1	Antibody Responses	138
7.3.1.1	Serum Antibody Response	138
7.3.1.2	Mucus Antibody Response	140
7.3.1.3	Gut Lavage Fluid Antibody Response	142
7.3.2	Clinical Signs	144
7.3.3	Gross Findings	146
7.3.4	Histological Analysis	149
7.3.5	Bacterial Isolation	156
7.4	Discussion	157
<b>8</b>	<b>GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS</b>	<b>162</b>
<b>BIBLIOGRAPHY</b>		<b>174</b>
<b>APPENDICES</b>		<b>189</b>
<b>BIODATA OF STUDENT</b>		<b>208</b>