

記錄 編號	6320
狀態	NC094FJU00105017
助教 查核	
索書 號	
學校 名稱	輔仁大學
系所 名稱	生命科學系
舊系 所名 稱	
學號	493546194
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論文 名稱 (中)	點帶石斑抗黏液病毒與免疫球蛋白重鏈基因在胚胎期之表現
論文 名稱 (英)	Expression of Epinephelus coioides Myxovirus Resistance (Mx) and Immunoglobulin M (IgM) heavy chain genes during development
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校內 全文 開放 日期	
校外 全文	

開放日期	
全文不開放理由	
電子全文送交國圖	
國圖全文開放日期	
檔案說明	
電子全文	
學位類別	碩士
畢業學年度	94
出版年	
語文別	中文
關鍵字(中)	點帶石斑 神經壞死病毒 抗黏液病毒基因 免疫球蛋白重鏈基因 整體原位雜交法
關鍵字(英)	Epinephelus coioides Neuron Necrosis Virus Mx gene Immunglobulin M Heavy chain gene whole-mount in situ hybridization
摘要(中)	<p>魚類先天性免疫與後天性免疫反應保護作用，是防禦外源病原感染的基礎，為了研究石斑魚免疫相關基因在發育早期的表現，我們首先以石斑魚腦細胞株 (GB) 進行神經壞死病毒之繁殖與純化，所得純化之神經壞死病毒顆粒進行紐西蘭大白兔免疫接種，製備出具有中和神經壞死病毒活性且高效價之多株抗體。抗神經壞死病毒多株抗體以被動免疫的方式稀釋 2,000 倍，浸泡中和沾黏在石斑魚受精卵卵鞘外之可能的感染源後，以 RT-PCR 追蹤受精卵以及孵化後之魚苗，證實無神經壞死病毒之感染後，培育魚苗作為研究材料，分析不同發育時期魚苗免疫基因之表現，本實驗室以點帶石斑 Mx 與 IgM 重鏈基因，分別做為先天性與後天性免</p>

	<p>疫基因標幟，並轉錄成 Digoxigenin-UTP 標定之 RNA 探針，進行整體原位雜交法；發現 Mx 基因轉錄在受精卵受精後第 21 (孵化前)至受精後第 39 小時 (孵化後第一天後期)有訊號表現，表現位置在胸腺與前腎，但在受精後第 51 小時 (孵化後第二天前期)訊號下降，而在 RT-PCR 的檢測中，則發現在受精後第 5 小時即可表現；IgM 重鏈基因轉錄在受精卵受精後第 21 小時 (孵化前)至受精後第 27 小時 (孵化後第一天前期)，有訊號表現且位置在胸腺，但在受精後第 39 小時 (孵化後第一天後期)訊號下降，而 RT-PCR 檢測則在受精後第 5 小時偵測到表現。此結果表示石斑魚先天性與後天性免疫基因在早期受精卵孵化至幼苗時期在淋巴器官內已開始基因表現。</p>
<p>摘要 (英)</p>	<p>Fish innate and adaptive immunity is a protective mechanism to defense foreign pathogens infection. In order to study the grouper immune-related gene expression at early stage, we used GB (grouper brain) cells to propagate and purify the NNV (Nervous Necrosis Virus) viral particles. The immunization inoculation of NNV viral particles into New Zealand rabbits produced a high titer anti-NNV polyclonal antibody, with the ability of neutralizing virus. To investigate the feasibility of passive immunity, we dilute the antibody in 2,000 fold in sea water and soaked the grouper eggs for 1 hour to disinfect the possible pathogens like NNV which may adhere on the surface of egg sheath. RT-PCR was then performed to trace the eggs and hatched out larvae. The larvae which was confirmed without NNV infection were analyzed the immune gene transcripts by using Epinephelus cocoidies Mx and IgM-heavy chain gene as innate and adaptive immune marker gene, respectively. These 2 genes were than transcribed to Digoxigenin labeled RNA probes for whole-mount in situ hybridization. The transcription of Mx gene showed signal at 21 hpf to 39 hpf in thymus, head kidney, and decreased at 51 hpf . RT-PCR can also detect the signal at 5 hpf. IgM-Heavy chain gene was transcribed at 21 hpf to 27 hpf in thymus, and the signal decreased at 39 hpf. RT-PCR can also detect the signal at 5 hpf. These results indicate the grouper innate and adaptive immune genes are transcribed in lymphoid organ during embryo to early larva stage.</p>
<p>論文 目次</p>	<p>謝誌 ----- I 目錄 --- ----- III 中文摘要 ----- ----- VII 英文摘要 ----- ----- IX 第一章 前言 ----- ----- 1 1. 石斑魚之簡介 ----- ----- 1 2. 神經壞死病毒之簡介與疾病症狀 ----- ----- 2 3. 硬骨魚之免疫系統 ----- 5 4. 抗黏液病毒之簡介 ----- 9 5. 免疫球蛋白 M 之簡介 ----- 11 6. 實驗目的 ----- ----- 12 第二章 材料與方法 ----- ----- 14 1. 生物性材料 ----- ----- 14 2. 神經壞死病毒之純化、蛋白質電泳分析與多株抗體 製備 --- 14 1. 藥品試劑 ----- 14 2. NNV 之</p>

純化 -----	17	3. NNV 蛋白質電泳分析 -----	
-----	20	4. NNV 之病毒效價 -----	
23	5. 不同 MOI 條件下 NNV CAV 之蛋白質電泳分析 -	24	6. 不同 MOI 與天數下 NNV RV 之 ELISA 分析 -----
24	7. 製備抗 NNV 多株抗體 -----		
25	8. 抗 NNV 多株抗體之 ELISA 分析 -----	26	
9. NNV 與抗 NNV 多株抗體之中和試驗 -----	27	10. 抗 NNV 多株抗體之西方墨點法 -----	28
11. NNV RV 之 ELISA 分析 -----	30	12. 石斑魚受精卵與魚苗之 RNA 抽取 -----	30
13. NNV 之 RT-PCR 分析 -----	31	3. 整體原位雜交法 -----	33
-----	33	1. 藥品試劑 -----	
-----	33	2. pBS-Mx2 質體之構築 -----	38
3. pBS-Mx2-5' 與 pBS-Mx2-3' 質體之構築 -----	39	4. DIG 標定 Mx2 RNA 探針之製備 -----	40
5. DIG 標定 Mx2-5' 與 Mx2-3' RNA 探針之製備 -----	41	6. DIG 標定 IgM Heavy chain RNA 探針之製備 -----	42
7. DIG 標定 RNA 探針之定量 -----	42	8. DIG 標定 RNA 探針之 RNA 電泳與轉印 -----	43
9. 石斑魚受精卵粉之製備 -----	45	10. 抗體以石斑魚受精卵粉進行前吸附作用 -----	46
11. 整體原位雜交法 -----	46	12. RT-PCR 偵測 Mx 基因之轉錄 -----	50
13. RT-PCR 偵測 IgM Heavy chain 基因之轉錄 -----	51	第三章 結果 -----	
52 抗神經壞死病毒多株抗體之開發 -----	52	1. NNV 之純化 -----	52
2. 找尋純化 NNV CAV 最佳條件 -----	52	3. 製備抗 NNV 多株抗體與西方墨點法確認 -----	53
4. 中和試驗確認抗 NNV 多株抗體之效價 -----	54	5. ELISA 分析抗 NNV 多株抗體之效價 -----	54
6. ELISA 分析在不同 MOI 與天數下 NNV RV 病毒量 -----	55	7. 田間試驗中抗 NNV 多株抗體之應用 -----	55
8. 探討點帶石斑 Mx 與 IgM Heavy chain 基因之表現 -----	56	1. 構築 pBS-Mx2 質體 -----	56
2. 構築 pBS-Mx2-5' 、 pBS-Mx2-3' 質體 -----	56	3. DIG 標定之 pBS-Mx2 、 pBS-Mx2-5' 、 pBS-Mx2-3' 與 IgM-H RNA 探針製備與確認 -----	57
4. 整體原位雜交法偵測石斑魚早期不同時期 Mx 與 IgM-Heavy chain 基因之 RNA 表現 -----	57	5. RT-PCR 偵測石斑魚早期不同時期 Mx 與 IgM-Heavy chain 基因之 RNA 表現 -----	59
59 第四章 討論 -----			
60 第五章 考文獻 -----	65	圖目次	
圖一 NNV CAV 的病毒帶 -----	74	圖二 SDS-PAGE 分析純化後之 NNV -----	75
圖三 SDS-PAGE 分析在不同 MOI 條件下純化出 NNV CAV 之差異 -----	76	圖四 西方墨點法確認第一次免疫接種 NNV 後的免疫反應 ----	77
圖五 西方墨點法確認第二次免疫接種 NNV 後的免疫反應 ----	78	圖六 西方墨點法確認第三次免疫接種 NNV 後的免疫反應 ----	79
圖七 西方墨點法確認第四次免疫接種 NNV 後的免疫反應 ----	81	圖八 ELISA 分析在不同 MOI 與天數下繁殖 NNV RV -----	83
圖九 光學顯微鏡觀察 NNV 在不同 MOI 感染 GB 細胞的 CPE 現象 -----			

----- 84 圖十 RT-PCR 檢測受精卵和孵化後之魚苗感染 NNV 之情形 -- 85 圖十一 構築 pBS-Mx2、pBS-Mx2-5'、pBS-Mx2-3' 質體 ----- 86 圖十二 RNA 電泳確認 DIG 標定 pBS-Mx2、pBS-Mx2-5'、pBS-Mx2-3' 與 IgM-H RNA 探針 ----- 88 圖十三 DIG 標定之 pBS-Mx2、pBS-Mx2-5'、pBS-Mx2-3' 與 IgM-H RNA 探針 ----- 89 圖十四 Dot blot 分析 DIG 標定 pBS-Mx2、pBS-Mx2-5'、pBS-Mx2-3' 與 IgM-H RNA 探針之濃度 ---- ----- 91 圖十五 石斑魚受精卵至魚苗生長圖 圖十六 整體原位雜交法偵測石斑魚受精卵早期不同時期 Mx RNA 之表現 ----- ----- 96 圖十七 整體原位雜交法偵測石斑魚受精卵早期不同時期 IgM Heavy chain RNA 之表現 ----- ----- 98 圖十八 RT-PCR 偵測石斑魚受精卵 Mx RNA 之表現 ----- 99 圖十九 RT-PCR 偵測石斑魚受精卵 IgM Heavy chain RNA 之表現 100 附圖目次 附圖一 pBluescript SK - 質體圖 ----- ----- 101 附圖二 斑馬魚胸腺與腎臟位置圖 ----- ----- 102

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論文
頁數

113

附註

全文 點閱 次數	
資料 建置 時間	
轉檔 日期	
全文 檔存 取記 錄	
異動 記錄	M admin Y2008.M7.D3 23:18 61.59.161.35