

白點症病毒結構性蛋白質VP15(ORF269)及VP32(ORF253)之特性分析

楊惠晴、張雲祥

E-mail: 345325@mail.dyu.edu.tw

摘要

本研究針對兩個白點症病毒結構性蛋白進行特性分析，分別為VP15 (WSSV-TW ORF269, GeneBank accession no. AF440570) 及VP32 (WSSV-TW ORF253, GeneBank accession no. AF440570)。基因表現時序分析結果表示VP15在白點症病毒感染中屬於早期基因，以酵母雙雜交篩選基因庫實驗顯示，VP15與VP19, VP26, VP95, VP51C, VP136, VP664, pmRACK1, F1ATP synthase beta subunit和VP15本身具有交互作用。另外對VP32所進行的酵母雙雜交實驗中發現VP32與VP187, VP19, VP60B, pmRACK1, F1ATP和pmCBP具交互作用，以西方墨點法分析VP32於病毒顆粒上定位結果顯示，VP32為一個外套膜蛋白；膜拓撲學預測分析進一步指出，VP32不具有穿膜區，同時其所在位置為病毒外套膜的外層。

關鍵詞：白點症病毒、VP15、VP32、結構性蛋白、蛋白質交互作用

目錄

封面內頁 簽名頁 中文摘要 iii 英文摘要 iv 誌謝 v 目錄 vii 圖目錄 xi 表目錄 xiii 1. 前言 1 1.1.白點症病毒 1 1.2.白點症病毒結構性蛋白質簡介 3 1.3.蛋白質之交互作用 4 1.4.研究目的 7 2. 材料方法 9 2.1.實驗流程-VP15 9 2.2.實驗流程-VP32 10 2.3.基因表現之分析 11 2.3.1.Total RNA 萃取 11 2.3.2.以DNaseI處理 11 2.3.3.反轉錄聚合反應 (Reverse transcription, RT) 12 2.3.4.聚合酵素鏈鎖反應 (Polymerase chain reaction, PCR) 12 2.4.蛋白質表現及特性分析 13 2.4.1.VP32重組蛋白質表現 13 2.4.1.1.少量細胞先驅測試 13 2.4.1.2.pET28b- VP32重組蛋白之水溶性測試 14 2.4.1.3.少量離心純化測試 15 2.4.1.4.抗體製備 15 2.5.病毒顆粒上之定位分析 17 2.5.1.白點症病毒純化 17 2.5.2.白點症病毒之抗VP32抗體西方轉漬分析 19 2.5.3.以不同之鹽濃度和Triton X-100分析VP32於白點症病毒顆粒上定位 20 2.6.VP32膜拓撲學(membrane topology)分析 20 2.6.1.VP32蛋白質親疏水性分析 20 2.6.2.VP32穿膜區預測 21 2.6.3.VP32在白點症病毒顆粒之膜拓撲學分析 21 2.7.VP15與VP32於酵母菌雙雜交篩選系統中與其他白點症病毒結構性蛋白質交互作用分析 22 2.7.1.VP15與VP32於酵母菌雙雜交篩選系統中自體激活測試載體構築 22 2.7.2.酵母菌轉染 22 2.7.3.自體激活測試 23 2.7.4.pGADT7酵母菌基因庫(yeast library)構築 23 2.7.5.pGBKT7-VP15與pGBKT7-VP32於酵母菌中蛋白質表現 23 2.7.6.pGBKT7-VP15與pGBKT7-VP32酵母菌基因庫篩選 24 2.8.VP32利用桿狀病毒表現載體系統生產重組VP32 25 2.8.1.質體DNA構築 25 2.8.2.重組BAC-1-FLAG-VP32之病毒生產 25 2.8.3.以溶菌斑試驗 (plaque assay) 進行BAC-1-FLAG-VP32重組病毒單株化 26 2.8.4.重組病毒力價分析 (TCID50 assay) 27 3.結果 28 3.1.VP15 (WSSV269) 28 3.1.1.基因表現時序分析 28 3.1.2.VP15篩選酵母菌基因庫與其他白點症病毒交互作用之分析 28 3.1.3.pGBKT7-VP15在酵母菌雙雜交系統中自體激活 (Autoactivation) 試驗 29 3.2.VP32(WSSV253) 30 3.2.1.VP32病毒顆粒定位分析 30 3.2.2.抗體製備 31 3.2.3.VP32病毒顆粒上定位分析 31 3.2.4.膜拓撲學分析 32 3.2.5.VP32篩選酵母菌基因庫與其他白點症病毒交互作用之分析 32 3.2.6.pGBKT7-VP32在酵母菌雙雜交系統中自體激活 (Autoactivation) 試驗 33 3.2.7.確認VP32於昆蟲Sf9細胞中表現 34 4.討論 36 5.結論 40 參考文獻 75 附錄 80 圖目錄 圖1.白點症病毒VP15基因mRNA轉錄表現時序分析 41 圖2.利用12%SDS PAGE分析經超高速離心機純化的白點症病毒顆粒 42 圖3.西方墨點法分析轉形於酵母菌中VP15表現 43 圖4.白點症病毒結構蛋白VP15在酵母菌雙雜交系統中自體激活試驗 44 圖5-1.酵母菌基因庫雙雜交系統之VP15與VP15、VP51C、VP26之交互作用 45 圖5-2.酵母菌基因庫雙雜交系統之VP15與VP664-11、VP19、VP95之交互作用 46 圖5-3.酵母菌基因庫雙雜交系統之VP15與VP136N、pmRACK1、pmCBP之交互作用 47 圖6.在Sf9昆蟲細胞內pDHsp70-V5-V5-VP15表現 48 圖7.白點症病毒VP32蛋白親水性、疏水性預測 49 圖8.VP32穿膜位置分析 50 圖9.重組VP32蛋白質表現時序分析 51 圖10.BL21(RIL)- pET28b-VP32重組蛋白質之純化，將誘導3小時菌液進行破菌 52 圖11.BL21(RIL)- pET28b-VP32重組蛋白質大量離心純化測試製備抗體蛋白濃度 53 圖12.VP32重組蛋白生產抗體第五次抗體測試血清與白點症病毒進行西方墨點法測試 54 圖13.西方墨點法分析VP32在白點症病毒顆粒上之定位分析 55 圖14.VP32蛋白質之病毒拓撲學分析 56 圖15.西方墨點法分析酵母菌中pGBKT7-VP32蛋白質表現 57 圖16.白點症病毒結構蛋白VP32在酵母菌雙雜交系統中自體激活試驗 58 圖17-1.酵母菌基因庫雙雜交系統之VP32與VP187N、VP19、VP60B之交互作用 59 圖17-2.酵母菌基因庫雙雜交系統之VP32與pmRACK1、pmCBP、F1ATP之交互作用 60 圖18.以西方墨點法與確認重組BAC-1-FLAG-VP32於昆蟲細胞Sf9表現 61 圖19.重組BAC-1-VP32 plaque assay挑選單株病毒株於昆蟲細胞Sf9表現以PCR於膠體電泳中挑選具表現基因 62 圖20.西方墨點法確認重組BAC-1-VP32以於昆蟲細胞Sf9表現plaque assay挑選單株病毒株 63 圖21.重組BAC-1-FLAG-VP32 plaque assay挑選單株病毒株#5限數稀釋法 (Limiting dilution) 感染Sf9昆蟲細胞後以PCR分析 64 圖22.酵母菌基因庫以酵母菌雙雜交篩選出具有交互作用蛋白質 65 表目錄 表1.VP15基因時序表現分析之特定核酸引

子 66 表2.構築於細胞中表現蛋白質載體引子 67 表3.構築於酵母菌中表現蛋白質載體引子 68 表4.構築於蛋白質中表現載體引子 69 表5.構築於酵母菌中表現蛋白質載體引子 70 表6.構築於桿狀病毒中表現蛋白質載體引子 71 表7.本研究中所參與建構之白點症病毒結構性蛋白質酵母菌雙雜交基因庫 72 表8.VP15於酵母菌雙雜交系統所篩選到之白點症病毒結構性蛋白質之分析表格 73 表9.VP15於酵母菌雙雜交系統所篩選到之白點症病毒結構性蛋白質之分析表格 74

參考文獻

- 1.周宗錄, 2007。蝦白點症病毒結構性蛋白VP51A(ORF294)特性分析。私立大葉大學分子生物科技學系碩士論文。
- 2.熊慧叡, 2010。白點症病毒結構性蛋白VP11(ORF394)之特性分析。私立大葉大學分子生物科技學系碩士論文。
- 3.趙梓霖, 2010。白點症病毒結構性蛋白VP38A(ORF314)之特性分析。私立大葉大學分子生物科技學系碩士論文。
- 4.Chang YS, Liu WJ, Lee CC, Chou TL, Lee YT, Wu TS, Huang JY, Huang WT, Lee TL, Kou GH, Wang AH, Lo CF A 3D Model of the Membrane Protein Complex Formed by the White Spot Syndrome Virus Structural Proteins. *PLoS one* 5:e10718
- 5.Chang PS, Lo CF, Wang YC, Kou GH(1996) Identification of white spot syndrome associated baculovirus (WSSV) target organs in shrimp, *Penaeus monodon*, by in situ hybridization. *Dis Aquat Org* 27:131-139
- 6.Chen LL, Leu JH, Huang CJ, Chou CM, Chen SM, Wang CH, Lo CF, Kou GH (2002a) Identification of a nucleocapsid protein (VP35) gene of shrimp white spot syndrome virus and characterization of the motif important for targeting VP35 to the nuclei of transfected insect cells. *Virology* 293:44-53
- 7.Chen LL, Wang HC, Huang CJ, Peng SE, Chen YG, Lin SJ, Chen WY, Dai CF, Yu HT, Wang CH, Lo CF, Kou GH (2002b) Transcriptional analysis of the DNA polymerase gene of shrimp white spot syndrome virus. *Virology* 301:136-147
8. Dhar AK, Dettori A, Roux MM, Klimpel KR, Read B(2003) Identification of differentially expressed genes in shrimp (*penaeus stylirostris*) infected with white spot syndrome virus by cDNA microarrays. *Archives of Virology* 148:2381-2396
- 9.Lotz JM, Browdy CL, Carr WH, Frelter PF, Lightner DV(1995) USMSFP suggested procedures and guidelines for assuring the specific pathogen status of shrimp broodstock and seed. In: Browdy CL, Hopkins JS (eds) *Swimming Through Troubled Water, Proceedings the Special Session on Shrimp Farming, Aquaculture '95*. World Aquaculture Society, Baton Rouge, Louisiana, USA, p 66-75
- 10.Lo CF, Kou GH,(1998) Virus associated white spot syndrome of shrimp in Taiwan: a review. *Fish Pathol* 33:365-371.
- 11.Lo CF, Peng CH, Chen SE, Hsu CH, Chiu HC, Chen YL, Chang YT, Liu CF, Su KF, Wang MS, Kou GH(1996a) Infection of white spot syndrome associated virus (WSBV) in cultured and wild-caught shrimps, crabs and other arthropods. *Dis Aquat Org* 27:215-225
- 12.Lo CF, Leu JH, Ho CH, Chen CH, Peng SE, Chen YT, Chou CM, Yeh PY, Huang CJ, Chou HY, Wang CH, Kou GH(1996b) Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. *Dis Aquat Org* 25:133-141
- 13.Liu WJ, Yu HT, Peng SE, Chang YS, Pien HW, Lin CJ, Huang CJ, Tsai MF, Huang CJ, Wang CH, Lin JY, Lo CF, Kou GH(2001) Cloning, characterization, and phylogenetic analysis of a shrimp white spot syndrome virus gene that encodes a protein kinase. *Virology* 289:362-377
- 14.Lightner DV, Hedrick RP, Fryer JL, Chen SN, Liao IC, Kou GH(1987) A survey of cultured penaeid shrimp in Taiwan for viral and other important disease. *Fish Pathol.* 22, 127-133
- 15.Lightner DV, Poulos BT, Redman RM, Mari J, Bonami JR(1992) New developments in penaeid virology: Application of biotechnology in research and disease diagnosis for shrimp viruses of concern in the Americas. In: Fulks, W., and Main, K. (eds) *Proceedings of the Asian Interchange Program Workshop on the Diseases of Cultured Penaeid Shrimp*, Asian Interchange Program, The Oceanic Institute, Oahu, HI, 233-253
- 16.Marks H, Mennens M, Vlaskovic JM, van Hulten MC(2003) Transcriptional analysis of the white spot syndrome virus major virion protein genes. *J Gen Virol* 84:1517-1523
- 17.Sanchez-Paz, A. White Spot Syndrome Virus: an overview on an emergent concern. *Vet Res* 41:43
- 18.Sangsuriya P, Senapin S, Lo CF, Flegel TW(2011) Co-interactive DNA-binding between a novel, immunophilin-like shrimp protein and VP15 nucleocapsid protein of white spot syndrome virus
- 19.Tsai JM, Wang HC, Leu JH, Hsiao HH, Wang AH, Kou GH, Lo CF(2004) Genomic and proteomic analysis of thirty-nine structural proteins of shrimp white spot syndrome virus. *J Virol* 78:11360-11370
- 20.Takahashi Y, Itami T, Kondo M, Maeda M, Fujii R, Tomonaga S, Supamattaya K, Boonyaratpalin S(1994) Electron microscopic evidence of bacilliform virus infection in kuruma shrimp (*Penaeus japonicus*). *Fish Pathol* 29:121-125
- 21.Tang X, Hew CL(2007) Expression, purification and crystallization of two major envelope protein from white spot syndrome virus. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 7:624-6
- 22.Tsai JM, Wang HC, Leu JH, Wang AH, Zhuang Y, Walker PJ, Kou GH, Lo CF(2006) Identification of the Nucleocapsid, Tegument, and Envelope Proteins of the Shrimp White Spot Syndrome Virus Virion. *J Virol.* 80:3021-3029
- 23.Tsai MF, Lo CF, van Hulten, Tzeng MC, Chou HF, Huang CM, Wang CJ, Lin CH, Vlaskovic JY, Kou G.H.(2000a) Transcriptional analysis of the ribonucleotide reductase genes of shrimp white spot syndrome virus. *Virology* 277:92-99
- 24.Tsai MF, Yu HT, Tzeng HF, Leu JH, Chou CM, Huang CJ, Wang CH, Lin JY, Kou GH, Lo CF(2000b) Identification and characterization of a shrimp white spot syndrome virus (WSSV) gene that encodes a novel chimeric polypeptide of cellular-type thymidine kinase and thymidylate kinase. *Virology* 277:100-110
- 25.Van Hulten MC, Reijns M, Vermeesch AM, Zandbergen F, Vlaskovic JM(2002) Identification of VP19 and VP15 of white spot syndrome virus (WSSV) and glycosylation status of the WSSV major structural proteins. *J Gen Virol* 83:257-265
- 26.Van Hulten MC, Westenberg M, Goodall SD, Vlaskovic JM(2000) Identification of two major virion protein genes of white spot syndrome virus shrimp. *Virology* 266:227-36
- 27.vlaskovic JM, Bonami JR, Flegel TW, Kou GH, Lightner DV, Lo CF, Loh PC, Walker PW(2005) Nimaviridae. In: VIIIth Report of the International Committee on Taxonomy of Viruses. (Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger, and L.A. Ball.) Elsevier. 32:187-192
- 28.Wang CH, Lo CF, Leu JH, Chou CM, Yeh PY, Chou HY, E TM, Chang CF, Su MS, Kou GH(1995) Purification and genomic analysis of baculovirus associated with white spot syndrome (WSBV) of *Penaeus monodon*. *Dis Aquat Org* 23:239-242
- 29.Witteveldt J, Vermeesch AM, Langenhof M, de Lang A, Vlaskovic JM, van Hulten MC(2005) Nucleocapsid protein VP15 is the basic DNA binding protein of white spot syndrome

virus of shrimp. Arch Virol. 150:1121-33 30.WuW, Wang L, Zhang X(2005) Identification of white spot syndrome virus (WSSV) envelope proteins involved in shrimp infection. Virology 332.2:578-83 31.Yang F, He J, Lin X, Li Q, Pan D, Zhang X, Xu X(2001) Complete genome sequence of the shrimp white spot bacilliform virus. J Virol 75:11811-11820 32.Zhang X, Huang C, Qin Q(2004) Antiviral properties of hemocyanin isolated from shrimp *penaeus monodon*. Antiviral Res 61.2:93-9