甲殼類白點病病毒檢測方法與動物感染模式之建立

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摘要

蝦類白點病病毒（White spot syndrome virus, WSSV）為危害台灣養殖業最重要之蝦類病毒性疾病之一。開發無白點病病毒感染蝦隻之種原技術為目前業界所需，而在飼料添加抗病毒劑即為其中一種方式，然如何評估其效力則需先建立適當之動物模式。本研究以 PCR、Real-time PCR 技術先建立偵測蝦類檢體白點病病毒核酸定性及定量方法；以免疫化學染色技術建立偵測組織內病毒分佈方法，再進一步將白蝦以注射及浸泡途徑人工接種種毒，建立蝦隻感染本病模式，作為未來種毒生產、動物攻毒及進一步評估抗病毒劑效力之方法。藉由上述所建立之方法，已穩定生產出高濃度且無其他病原汙染之種毒，並建立白蝦以注射及浸泡途徑之動物感染模式且確認其 LD_{50} 及 LT_{50}。藉由免疫化學染色試驗結果顯示，本所製劑系先前開發之抗蝦類白點病病毒卵黃抗體對感染蝦組織中 WSSV 抗原具特異免疫結合活性，未來可供進一步評估此抗體保護蝦隻抗 WSSV 感染之效力，也可用來開發檢測 WSSV 抗原快速檢測試劑之用。

Establishment of crustacean white spot syndrome virus detection method and animal infection model

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Abstract

Shrimp white spot syndrome virus (WSSV) is one of the most important shrimp viral diseases that harmful to the aquaculture industry in Taiwan. The development of the technology for establishment of wssv free shrimp is currently needed by the industry. By adding antiviral agents to shrimp feed is one of the resolutions, but it requires establishing an appropriate animal model to evaluate its effectiveness. In this study, PCR and Real-time PCR were used to establish a qualitative and to quantitative method for wssv nucleic acid detection in shrimp specimens. Immunocytochemistry technique was used to establish a method for detecting virus distribution in tissues. Then, white shrimps were artificial inoculation of seed virus by injection and soaking method to establish an animal model of wssv infection. They can further apply for production of seed virus, challenge of experiment shrimp and the effectiveness evaluation of antiviral agent methods. Through the above-established method, a high-concentration seed virus without contaminating other pathogens has been stably produced, and an animal infection model of white shrimp by injection and soaking routes has been established and its LD_{50} and LT_{50} have been confirmed. The results of immunochemical staining experiments show that the previously developed anti-wssv egg yolk antibody has specific immunological binding activity to the wssv antigen in the infected shrimp tissues. This antibody can be further evaluated the effectiveness in the future to protect shrimp against from wssv. It can also be used to develop rapid detection reagents for wssv.