螯蝦瘟之分子生物學診斷研究與 2014 年 6 至 11 月電顯室工作報告

生物研究組
吳介豪 助理研究員

摘要

螯蝦瘟(crayfish plague)為造成螯蝦大量死亡之真菌性傳染病，過去由北美螯蝦帶原傳入歐洲造成歐洲原生螯蝦大量死亡。2013 年底我國養殖澳洲螯蝦(*Cherax quadricarinatus*)首次發生疫情，診斷為 *Aphanomyces astaci* 感染。為建立螯蝦瘟之診斷流程，收集苗栗、彰化及屏東之螯蝦檢體，進行病理學檢查及特殊染色，可見黴菌菌絲侵入尾部及步足關節甲殼層，產生局部肉芽腫；參考 OIE 公告方法進行黴菌分離，結果未分離出 *A. astaci*。分子診斷以 ITS (internal transcribed spacer)及 chitinase 基因片段進行 PCR 增幅，PCR 產物序列經 BLAST 比對為 *A. astaci*，並以 ITS 片段之 PCR 與 real-time PCR 檢測比較極限值。為瞭解螯蝦瘟之型別，參考 microsatellite genotyping 方法選取兩段基因片段進行增幅及大小分析，推測為 D 基因型。本試驗成功建立螯蝦瘟之病理學及分子診斷流程。

2014 年 6 月至 11 月電顯室負染色檢體總計 217 件，包含禽類 39 件，草食動物 61 件，水產動物 32 件，豬 42 件，其他 43 件，其中 45 件檢體有檢出特異性病原顆粒。

Molecular Diagnosis of Crayfish Plague and Periodic Report of Electron Microscopy from June to November, 2014

Chieh-Hao Wu

Abstract

The oomycete *Aphanomyces astaci* is the etiology of crayfish plague and has devastated native crayfish populations across Europe due to introducing the infected North American crayfish. In the end of 2013, the first outbreak of *A. astaci* infection in cultivated Australian crayfish (*Cherax quadricarinatus*) was diagnosed in Taiwan. The crayfish specimens were collected from Miaoli, Changhua and Pingtung to establish a diagnosis procedure for crayfish plague. The pathological examinations with special staining revealed penetration of hyphae and granulomatous inflammation in chitinous layer of uropod and articulation. *Aphanomyces astaci* was not successfully isolated from these specimens based on OIE Manual of Diagnostic Tests for Aquatic Animals. For molecular diagnosis, PCR amplications of internal transcribed spacer (ITS) and chitinase gene fragments were performed and the product sequences analyzed by BLAST showed high identities with *A. astaci.* In addition, detecting limits between PCR and real-time PCR targeting ITS fragment were compared. By microsatellite genotyping method, genotype D was assumed according to the size of two gene fragments. This study established pathological and molecular diagnoses of crayfish plague.

A total of 217 samples was collected for electron microscopy by negative stain from June to November in 2014, including 39 samples from poultry, 61 samples from herbivores, 32 samples from aquatic animals, 42 samples from swine, and 43 samples for pathogen observation. Forty-five of those specimens were observed with specific viron/bacteria.