幼蟲芽孢桿菌對蜜蜂的致病力與防治法

Pathogenicity and Conurol of Bacillus larvae in Honey Bees

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[摘要]

人工接種幼蟲芽孢桿菌 (*Bacillus larvae*) 孢子於西方蜂 (*Apis mellifera*) 1 日齡工 蜂幼蟲, 罹病幼蟲進入封蓋期 (capping stages) 後才會呈現典型美洲幼蟲病 (American foulbrood, AFB) 的病徵, 高劑量的感染會造成部分幼蟲 (32.2% -46.3%) 無法進入封蓋期。病原孢子對此齡幼蟲有很高的致病力, LD50 及 LD95 分別為 21 個孢子與 442 個孢子；而且病原體的致病力並無季節性的差異。相對於 1 日齡幼蟲的高致病力, 此病原孢子對西 方蜂 2 日齡幼蟲的致病力大為降低, 接種高劑量孢子 (4.5×10^4 spores/larva) 只有 37.2 % ±3.4%的死亡率。幼蟲芽孢桿菌對西方蜂 3 日齡幼蟲的致病力極低, 接種同一劑量孢子仍無罹病蟲體的出現。接種高劑量幼蟲芽孢桿菌孢子於東方蜂 (A. cerana) 幼蟲, 病原 體對 1 日齡及 2 日齡幼蟲才具有致病力並呈現如西方蜂之 AFB 病徵。東方蜂 1 日齡幼蟲對此病 原的感受性 (susceptibility) 比同日齡的西方蜂低, 接種相當於西方蜂 LD95 劑量的死亡率只有 47.1%；2 日齡的感受性也低於西方蜂, 接種相同高劑量 (4.5×10^4spores/larva) 的死亡率只有 23.3 ±5.0%, 顯示東方蜂對 AFB 具有很高的抗性。東方蜂群自然發生 AFB 的 情形極低, 可能的原因包括：(1) 幼蟲對病原體的感受性較低, 而且此種幼蟲本身的抗性機制表現於腸道中, 與幼蟲血體腔 (haemocoel) 的免疫系統無關。(2) 高達 1.1 -82.2% 的感染幼蟲於封蓋前即被東方蜂群的清潔行為移除, 明顯降低罹病蟲體形成孢子污染蜂群的程度。幼蟲芽孢桿菌對羥四環素 (oxytetracycline, OTC) 非常敏感, 室內培養基試驗 顯示 OTC 對營養體 (vegetative cells) 的最低抑制濃度 (MIC) 僅 0.016 μg/ml, 但不具 殺滅孢子的效果。田間試驗的結果顯示西方蜂群餵飼 1 次含 125 mg OTC 的糖漿, 可完全抑制 AFB 的發生至少達 9 日 (施藥前 2 日＋施藥後 7 日)；50 mg OTC 劑量則藥效至少達 3 日(施藥後)。台灣產蜂膠乙醇萃取物於室內培養基試驗也顯示具有良好的抗生作用, 對幼蟲芽孢桿菌營養體的 MIC 為 3.125 μg/ml, 但蜂群間隔 3 日連續餵飼 2 次含 400 mg 蜂膠萃取物的糖漿, 卻無法有效抑制 AFB 的發生。輕微感染 AFB 的西方蜂群 (病徵數＜50), 可藉換箱處理 保留成蜂, 新蜂群至少可達 15 週不會再發生 (recurrence) AFB；嚴重感染的蜂群(病徵 數＞500), 蜂群必須先經換箱處理, 並於換箱 5 日後餵飼 1 次含 125 mg OTC 的糖漿, AFB 才不會於 15 週內再發生。蜂群餵飼 OTC 糖漿後, 必須停止生產 1 個月, 而且恢復生產前需取出儲蜜, 以避免 OTC 殘留於蜂產品。實驗室內人工飼育西方蜂 1 日齡工蜂幼蟲至羽化成蜂, 羽化率為 55.4 ±8.4%, 利用此人工飼育技術, 添加 5 -150 ppm OTC 或 10 -500 ppm 蜂膠萃取物於食物中, 評估兩者對幼蟲發育的影響。食物中含 25 ppm OTC 或 100 ppm 蜂膠萃取物對幼蟲的生長發育與存活率並無顯著的影響, 但高於此濃度則幼蟲呈現生長延遲或存活率減 低的現象。另於食物中

加入 4.5×10^5 spores / ml 的幼蟲芽孢桿菌和 0.2 - 25 ppm 的 OTC 或 25 -100 ppm 蜂膠萃取物, 以探討兩者對 AFB 的抑病力。OTC 處理除 0.2 ppm 出現 1%的罹病個體, 其餘濃度皆可完全抑制 AFB 的發生, 顯示 OTC 確可有效防治 AFB；但添加 100 ppm 蜂膠萃取物仍無法有效抑制。

關鍵字: 幼蟲芽孢桿菌 Bacimlus larvae
美洲幼蟲病 American foulbrood
西方蜂 Apis melligera
東方蜂 Apis cerana
致病力 pathogenicity
防治 Control

【摘要】

Pathogenicity of Bacillus larvae spores to 1-day-old larvae, Apis mellifer a, was determined by experimental infection in Taiwan. The results showed no seasonal difference in mortality of larvae. Infected individuals showed AFB signs in the capping stage, while 32.2% - 46.3% died in the larval stage resulted from heavy infections. The 1-day-old larvae are highly susceptible toB. Larva e, LD50 and LD95 are 21 spores and 442 spores, respectively. In contrast, the mortality of 2-day-old larvae to B. larvae was much lower than that of 1-day-old larvae. The mortality of 2-day-old larvae was 37.2% ±3.4% after 4.5×10^4spores/larva inoculation, while 3-day-old larvae were not susceptible to thispathogen even at the same dose. By experimental infection with B. larvae spores to native Asian honeybee (A. cerana), 1-day-old and 2-day-old larvae were susceptible to this pathogen, and showed the AFB symptom like the infected European honeybee individuals. However, high tolerance of A. cerana compared with that of A. mellifera was found. Results showed that mortality of 1-day-old larvae of A. cerana was 47.1%±10.2% after 442 spores/larva (LD95 of A. mellifera) inoculation, and mortalityof 2-day-old larvae was 23.3% ±5.0% after 4.5×10^4 spores/larva inoculation. Natural outbreak of AFB occurred rarely in Asian honeybee colonies, the considerable factors may consist of : (1) less susceptibility in A. cerana larvae, and their resistant mechanism may be relation to midgut systems, not toimmune systems of pupal haemocoel. (2) most of infected larvae (61.1% -82.2%) were ejected in the larval stage by the hygienic behavior of A. cerana, and thatof decreased spore contamination in their hive.

In vitro tests, oxytetracycline (OTC) showed the highly inhibitory effect Onvegetative cells of B. larvae (MIC = 0.016 μg/ml), but no sporicide

effect. In field tests, honeybee colonies were medicated with OTC syrup to determine thei reffectiveness on young larvae prevention of AFB. Results showed 2 doses of OTC syrup, 125 mg/colony and 50 mg/colony, prevent AFB signs for a period of, at least, 9 days and 3 days, respectively. The ethanol extracts of Taiwanese propolis also showed a good inhibitory effect on vegetative cells of B. larvae (MIC = 3.125μg/ml) in vitro, while colonies medicated twice with 400 mg/colony treatments only had a limited effect in field tests. Colonies with a light AFB infection treated with hive replacement recovered from the disease and no AFB recurrence in a investigated period of 15 weeks. Beside the hive replacement,colonies with a heavy infection should be medicated additionally with 125mg of OTC on the 5th day post-replacement. OTC medication colonies should be stopped harvesting for a month. Honey stored during medication periods in combs should be removed before another harvests. An artificial method was developed for rearing worker honeybee (A. mellifera) larvae to the adult stage in the laboratory . This method subsequently was used in studying the effects of OTC and propolis extracts on larval growth and development. A general dose response was observed in mortalities of larvae fed diets supplemented with 60 ppm to 150 ppm OTC or 500 ppm propolis extracts. At 25 ppm OTC or 100 ppm propolis extracts in diets, larval and postdefecation mortalities, and larval growth rateswere similar to the controls, while higher than these doses retarded larval growth and caused higher mortality. When fed with 0.2 ppm, OTC effectively reduced larval and postdefecation mortalities of larvae inoculated with4.5×10^5 spores/ml. However, fed even up to 100 ppm , propolis extracts could not prevented the AFBinfection. It revealed that OTC effectively protected young larvae from B. larvae infection, while propolis extracts did not.