行政院國家科學委員會補助專題研究計畫成果報告

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※ 建立我國植物保護生物製劑產業有關關鍵生物技術與資源之發展應用： ※

※ *Bacillus* 幾丁質分解酵素基因 ※

※ 在 *Pseudomonas* 根圈細菌之表現分析 ※

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執行期間：2000 年 08 月 01 日 至 2001 年 07 月 31 日

子計畫主持人：陳昭瑩

執行單位：臺灣大學植物病理學系

中 華 民 國 九十 年 十 月 二十八 日

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一、中 文 摘 要

本研究自植物根圈中篩選對植物病原真菌具有拮抗性的螢光假單胞細菌，並導入芽孢桿菌屬細菌之幾丁質分解酵素基因，希望藉幾丁質分解酵素與抗生物質的協力作用來增加拮抗性螢光假單胞細菌對土壤傳播性植物病原真菌的抗菌能力。自植物根圈分離出 36 株螢光假單胞細菌，在平板上與白絹病菌對峙培養，各菌株表現不同程度的抗生作用。在 7 個抗生能力較強的菌株中，有 3 個菌株能降低盆栽菜豆受到白絹病菌感染的程度。將帶有芽孢桿菌屬細菌幾丁質分解酵素基因的質體 pMMST2000 以三親本雜交方式送入菌株 FP-16 中，轉形菌株產生的幾丁質分解酵素可轉移至細菌體表。盆栽生物防治試驗結果顯示野生菌株 FP-16 與轉形菌株 FP-16(pMMST2000)均能降低菜豆受到白絹病菌感染的程度，且轉形菌株有較強的防治效果。

關鍵詞：幾丁質分解酵素、幾丁質分解酵素基因、根圈細菌、生物防治

Abstract

The goal of this study is to screen fluorescent *Pseudomonas* antagonists from rhizosphere and construct a *Bacillus* chitinase gene-containing *Pseudomonas* strain. Based on the synergism of chitinase and antibiotic, the biocontrol activity can be enhanced. Thirty-six strains of fluorescent pseudomonads were isolated from plant rhizosphere. In the dual culture assay with *Sclerotium rolfsii*, different strains of fluorescent pseudomonads showed different antifungal activities. Among seven antagonistic *Pseudomonas* strains which showed higher antagonistic activity, three could decrease the disease incidence of bean by seed-coating treatment. Then, the *chiA*-containing pMMST2000 was transferred into *Pseudomonas* strain FP-16 by triparental mating. The chitinase produced in the

transformant was located in the outer membrane. The pot assay of biocontrol showed that both strain FP-16 and FP-16(pMMST2000) reduced the disease severity of bean infected by *Sclerotium rolfsii* and FP-16(pMMST2000) showed better biocontrol activity.

Keywords: chitinase, chitinase gene, rhizobacteria, biocontrol

二、緣由與目的

許多 *Pseudomonas* 根圈細菌能夠纏聚並長期存活於植物根圈，甚至能進入植物體中，能促進植物生長，並防治多種土媒病害(Bagnasco *et al.*, 1998; Botelho *et al.*, 1998; Brooks *et al.*, 1994; Freitas and Germida, 1992; Schisler *et al.*, 2000)。已知拮抗菌之抗菌原理與幾丁質分解酵素的產生及作用有關(Inbar and Chet, 1991; Kobayashi *et al.*, 1995; Nielsen *et al.*, 1998)。將幾丁質分解酵素基因轉殖到 *Pseudomonas* 根圈細菌中也可增加對土壤病原真菌的防治效果(Downing and Thomson, 2000; Koby *et al.*, 1994; Sundheim *et al.*, 1988)。如 Downing 及 Thomson (2000)將 *Serratia marcescens* 的幾丁質分解酵素基因 *chiA* 導入 *Pseudomonas fluorescens* 中，轉形菌株能有效地降低 *Rhizoctonia solani* 在菜豆上的危害。由於抗真菌物質與幾丁質分解酵素對真菌之抑制作用具有協力作用，只要微量濃度，即比單獨高濃度施用的效果更好(Lorito *et al.*, 1994, 1996; Schirmbock *et al*; 1994)，故可期望以幾丁質分解酵素促進拮抗菌株的抗菌能力。本研究嘗試將革蘭氏陽性細菌芽孢桿菌屬細菌的幾丁質分解酵素基因導入具有拮抗能力的螢光假單胞細菌中，測試轉形菌株對病原真菌拮抗能力及生物防治效果的影響。

三、結果與討論

從植物根部共分離並純化 36 株能在 King's B medium 上產生螢光色素的細菌，將這 36 株細菌與白絹病菌對峙培養，由抑制圈大小測知 36 個螢光假單胞細菌分離菌株具有拮抗能力。將其中 7 個螢光假單胞細菌分離菌株處理菜豆種子後，於盆栽中進行抗白絹病菌的測試。以高濃度菌液處理，以 FP-16 處理的菜豆種子的罹病率最低。以螢光基質分析法測定，FP-16 並不會產生幾丁質分解酵素。

將含芽孢桿菌屬細菌幾丁質分解酵素基因的質體 pMMST2000 以三親本雜交方式導入 FP-16 菌株中，在含 IPTG 的 trypan blue-glycol chitin 培養基上篩選轉形菌株，獲得菌株 FP-16 (pMMST2000)。以螢光分析法測得幾丁質分解酵素比活性為 0.882 μmole/min/mg protein。培養在已塗抹 IPTG 的 colloidal chitin 培養基上，於 28 ℃下培養六天後可觀察到轉形菌株 FP-16(pMMST2000)在菌落周圍形成明顯的透明圈。

將轉形菌株 FP-16(pMMST2000)與野生型菌株 FP-16 覆於菜豆種子上，種植在含白絹病菌菌核的盆土中，八天後觀察菜豆植株的存活率，均比罹病對照組高，顯示二菌株對白絹病具有生物防

治的能力，能使菜豆存活率增加 30%以上。菜豆種子包覆 FP-16(pMMST2000)的處理組，植株的存活率較野生型菌株處理組稍高。為顧及含 pMMST2000 的 FP-16 細菌比例在不含抗生素的條件下可能逐漸降低，而影響抗菌的效果；及在土壤環境中，FP-16(pMMST2000)幾丁質分解酵素基因的表現不如預期，將使幾丁質分解酵素基因插入螢光假單胞細菌染色體 DNA，使之穩定地存在於細菌菌體內，來提高幾丁質分解酵素促進拮抗作用的效果。此外根圈細菌也可能促進植物防禦能力的增加(Chen *et al.*, 2000; Wei *et al.*, 1996)，故可觀察地上部病害的防治效果。

四、成果自評

本研究完成階段性之系列試驗工作，如遺傳工程菌株之建構。具有拮抗能力螢光假單胞細菌之篩選也有初步的結果，但未來在不同植物的應用，適用菌株可能要重新篩選。針對 FP-16 菌株在菜豆上之病害防治試驗需補強，考慮擴展至其他土壤病原；防治原理除幾丁質分解酵素與抗生素之協力作用外，也考慮擴及對葉部病害抗病能力之誘發。

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