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促進植物生長之根棲細菌 *Streptomyces* sp. RS70 誘導番茄對青枯病之系統性抗性

鄧雅靜[1]　曾國欽[1]　徐世典[1,2]

[1] 台中市國立中興大學植物病理學系

[2] 聯絡作者，電子郵件：sthsu@mail.nchu.edu.tw；傳眞：+886-4-22877585

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摘　要

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Streptomyces sp. RS70 爲一可促進番茄生長之根棲細菌，且以種子被覆或幼苗澆灌處理具有防治番茄青枯病之效果，但在培養基上無抑制青枯病菌之能力。爲瞭解 RS70 之防病機制，本研究探討其誘導系統性抗病之可能性。將 RS70 以注入法處理番茄頂葉後，於第七天再由根部澆灌接種青枯病菌 PS152 時，青枯病之發病指數顯著低於頂葉未經 RS70 處理之對照組，此結果表示 RS70 可能誘導植株之抗性而降低發病程度。番茄頂葉經 RS70 注入處理後，至少需要六天才能有效誘導對青枯病之抗性，但此抗性僅持續數天。北方雜合分析發現，RS70 之處理可誘發未處理葉片之致病過程相關蛋白基因 *PR-1* 之表現，在測試 10 天期間內，其 mRNA 表現量隨處理天數之增加而增多，至第八天達最高峰 (較處理後 0 時增加約 2.3 倍)，隨後則略爲降低。RS70 不論以注入處理於頂葉或澆灌處理於根部後，皆能在未處理之葉片內偵測到 *PR-1* mRNA 之累積，顯示其誘發 *PR-1* 基因表現可向上或向下移行。本研究另以青枯病菌 PS152 之一株無致病力突變株測定時，發現其亦可誘導抗性並誘發 *PR-1* 基因之表現。

關鍵詞：根棲細菌、誘導抗病性、青枯病、番茄、*PR-1* 蛋白基因

緒　言

微生物防治植物土壤傳播性病害之可能機制包括養份[8] 或感染位置[25] 之競爭、產生嵌鐵物質[18, 19] 或抗生物質[11, 50]，此外誘導植物產生系統性抗病，亦爲生物防治的另一種機制[44]。

植物受到生物或非生物適當刺激後有時可提升其防禦能力，而能抵抗多種眞菌、細菌或病毒所引起之病害[13, 38, 44]。植物可藉由病原菌或弱病原菌之局部感染 (造成壞疽病徵) 而誘導抗病性之發生，進而抵抗其後所感染的多種病原菌[38]，抗性起初僅表現於初次被感染部位，但其後可由最初位置系統性移動到植株其他組織內，甚至延伸至根部[12, 39]，此種型式之抗病性常被稱爲後天系統性抗病 (systemic acquired resistance, SAR)。然而，促進植物生長之根棲細菌 (plant growth promoting rhizobacteria, PGPR) 雖爲腐生菌不會造成任何感染，但亦能誘發抗病性[23, 27, 44, 45]，爲與 SAR 作一區分，此種抗性常被稱爲誘導系統性抗病 (induced systemic resistance, ISR)[30]。SAR 首先是在煙草受到菸草嵌紋病毒 (tobacco mosaic virus) 局部感染之研究上而被發現[34]。隨後許多研究證明 SAR 與植物早期增加內部合成之水楊酸 (salicylic acid, SA) 含量有著密切關係[24, 26, 37, 48]，SA 的累積量具有調控 SAR 反應之決定性。又帶有 *nahl* G 基因之轉型植株，在受到病原菌感染後由於無法累積 SA，因而不能誘導 SAR 的產生[7, 10]。此外，SAR 可藉由活化所謂的 SAR 基因群來定性[49]，其中包括轉譯致病過程相關蛋白質 (pathogenesis-related proteins, PRs)[1, 43]，其通常作爲誘導 SAR 產生之標誌 (maker)。化學物質如水楊酸 (salicylic acid, SA) 處理植物後，除可誘導植物產生 SAR 外，常伴隨有 PRs 的累積[37, 46, 51]。因此，SA 的累積和 PR 蛋白質的過度表現爲

誘導 SAR 產生所必需。而 PGPR 所誘導之系統性抗病，ISR，多與 SA 或 PR 蛋白質之累積無直接關聯性，例如，*Pseudomonas fluorescens* strain WCS417r 誘導蘿蔔表現 ISR 時並無累積 PR 蛋白質[14, 15]，在誘導阿拉伯芥 (*Arabidopsis*) 之抗性時，此菌株與活化 PR 基因和SA之累積量亦無關[30, 31, 47]，意即其經由與 SA 無關之訊號路徑所調控。又如 *Serratia marcescens* 誘導胡瓜或煙草表現之 ISR[32] 與 *Bacillus pumilus* SE34 和 *Pseudomonas fluorescens* 89B61 誘發番茄對晚疫病菌產生之系統性抗性[52]，皆與 SA 的產生無關。但亦有例外，如 *P. fluorescence* strain CHA0 誘導煙草產生 ISR 時，與 PR 蛋白質之累積有著關聯性[27]，由此推測腐生性根棲細菌所誘導之 ISR 與病原菌誘導之 SAR 可能有分享著相似之機制。

由番茄根圈篩選獲得的 *Streptomyces* sp. RS70 爲一株 PGPR 菌株，具有促進番茄生長之效果[41]。此菌株亦可減少青枯病之發病程度，但在人工培養基上並無抑制青枯病菌之能力[41]。本研究之目的即在探討 *Streptomyces* sp. RS70 菌株之防病機制是否與誘導抗病性有關。

材料與方法

細菌菌株

Streptomyces sp. RS70 爲本研究室篩選所得對番茄生長具有促進作用及對青枯病具有防治效果之 PGPR 菌株[41]，*Ralstonia solanacearum* PS152 爲本研究室保存之番茄青枯病菌菌株，而由 PS152 菌株衍生之四株無致病力突變菌株 (PS152E、152H、152K 及 152R) 係由林宜賢提供。這些突變菌株爲 PS152 菌株之自然突變菌株，在多次培養於 TZC (tetrazolium chloride agar) 培養基[17] 平板上觀察到紅色圓形而外圍不具有白色流質狀之菌落而得，並以青枯病菌之專一性引子對 (Au759/Au760) 確認其爲青枯病菌[29]，再經番茄莖部穿刺接種試驗，確認其爲無致病力菌株。

接種源之製備

Streptomyces sp. RS70 菌株培養於營養減半之 PDA (每公升蒸餾水添加 24 克 Difco potato dextrose broth 及 12 克 agar) 培養基平板上，於 30℃培養 3 天後，以滅菌過之棉花棒於無菌水浸潤後，沾取菌落塗抹於馬鈴薯蔗糖洋菜培養基 (potato sucrose agar, PSA, 含馬鈴薯 200 克, 蔗糖 20 克, 瓊脂 12克, 蒸餾水 1000 毫升) 平板，再於 30℃培養 3 ~ 4 天後，懸浮於含 0.005% Tween 20 之無菌蒸餾水中，以 Spectronic 70 spectrophotometer (U-2000, Hitachi) 在波長 620 nm 下，調整 OD 值爲 0.4，此懸浮液細菌濃度約爲 10^8 cfu/ml，供作誘導抗病接種試驗用。青枯病菌 PS152 與其衍生之無致病力菌株分別劃線於 TZC 培養基[17] 平板上，於 30℃培養 2 ~ 3 天後，以滅菌過之棉花棒經無菌水浸潤後，分別沾取流質不規則圓形，中間爲粉紅色，外圍乳白色，具有致病力 (virulent) 之菌落，及紅色圓形而外圍不具有白色流質狀之無致病力 (avirulent) 菌落，塗抹於 523 培養基[16] 平板，於 30℃培養 24 ~ 48 小時後，將細菌懸浮於無菌蒸餾水中，調整其 OD 值爲 0.3，此懸浮液濃度約爲 10^8 cfu/ml，供作接種試驗用。

誘導抗病試驗

爲測試 *Streptomyces* sp. RS70 或青枯病菌 PS152 無致病力突變菌株能否誘導抗病性，將其接種於番茄頂端葉後，再接種具致病力之青枯病菌於根系上，使誘導者與病菌隔開，以排除二者間抗生及競爭的可能性。詳細方法爲將番茄 (雙福品種) 種子播植於裝有 finnpeat 泥炭土 (KEKKILÄ，芬蘭) 之保麗龍穴盤中 (12×20 孔，每一孔爲 2.5×2.5 公分)，在日溫 30℃，夜溫 25℃，光照 12 小時之生長箱內育苗 15 天後，再移植至含 finnpeat 泥炭土之黑色塑膠軟盆 (直徑 9 公分) 內，每盆移植一株幼苗，在同一生長箱中生長 15 天，以注入滲透法 (3) 將上述製備之 *Streptomyces* sp. RS70 懸浮液 (10^8 cfu/ml) 或 PS152 無致病力突變菌株懸浮液 (10^8 cfu/ml) 0.1 ml 注入最頂端已展開之第一或第二葉片內，對照組則分別以 0.005% Tween 20 或水處理，7 天後再將青枯病菌 PS152 懸浮液 (10^6 cfu/ml 或 10^5 cfu/ml) 由莖基部澆灌於盆內，每盆澆灌 70 ml，置於同一生長箱中觀察青枯病發生程度。每個處理 10 盆，試驗重覆二次。發病等級[41] 區分爲 0 級：無病徵；1 級：一葉片部分萎凋；2 級：一葉或二葉萎凋；3 級：三或更多葉片萎凋；4 級：全部葉片萎凋及 5 級：植株枯死。而發病指數則以 [Σ(發病等級×該發病等級株數) / (5×總株數)]×100% 計算。

誘導抗病所需之時間

依上述方法將 RS70 懸浮液 (10^8 cfu/ml) 注入番茄葉片內後於第 1、2、3、4、5、6、7、8、9 及 10 天分別將青枯病菌 PS152 懸浮液 (10^5 cfu/ml) 澆灌至盆內，放置於生長箱中觀察青枯病病勢進展。每個處理10盆，試驗重覆二次。

番茄葉片總量 RNA (total RNA) 之抽取

將收藏於 -80℃ 冷凍櫃中之番茄葉片，依據Napoli 等人[28] 之方法進行總量 RNA (total RNA) 的抽取。抽取之 RNA，稀釋 50 倍後測其 O.D.值 (DU series 500 Spectrophotometer, Beckman Instruiments Inc., CA)，計算A_{260}/A_{280} 比值及估算 RNA 的濃度，以評估其質與量。

番茄 *PR-1* 基因片段之增幅與探針之製備

聚合酵素連鎖反應 (polymerase chain reaction, PCR) 增幅 *PR-1* 基因片段所用之引子對，5`-CCCGAGCACAAAACTATGCC-3` / 5`-TGTCCGATCCAATTGCCTAC-3`，爲由 GeneBank accession number Y08804 核酸序列 (*PR1b1* 序列)[42] 設計而來[6]， PCR 反應液[22] 中並以由帶有 *PR-1* 基因質體之選殖菌株[53] 純化出之 *PR-1* 基因片段作爲模版 DNA。PCR 於 Peltier thermal cycler PTC200 (MJ research Inc., MA) 迴旋循環器中進行反應，其增幅條件爲以步驟 1：94℃，5 分鐘，1 個循環；步驟 2：94℃，1 分鐘；50-60℃，30 秒；72℃，1 分鐘；30 個循環；步驟3：72℃，10 分鐘，1 個循環。增幅後之產物以水平式電泳進行分析，再利用 Gel Elution Kit (GeneMark Technology Co., Taiwan) 回收膠體上 *PR-1* 基因片段，以製備探針。探針依據 Feinberg and Vogelstein[9] 之逢機引子反應法 (random primer reaction) 製備，在反應過程中添加 α-^{32}P -dATP 作爲標定物質，並以 0.5 M EDTA (pH 8.0) 終止反應後，隨即以 Sephadex G-50 管柱 (column) 純化探針。Sephadex G-50 管柱之製備方法如下：首先將 Sephadex G-50 溶於 TE 緩衝液 (buffer) 中，取 1 ml 含有 Sephadex G-50之溶液至微量離心管，管底部戳洞並填充玻璃珠，以 4000 g 離心 5 分鐘後，再以 100 μl STE 緩衝液 (200 mM NaCl，50 mM Tris-HCl pH 8.0，10 mM EDTA) 4000克離心 5 分鐘2 次，然後加入含^{32}P 標定之核酸探針於Sephadex G-50 管柱中，以4000g 離心5 分鐘即得純化探針。

北方雜合反應

根據Tan[40] 所述之方法進行番茄葉片 total RNA 之電泳分析及核酸探針之雜合反應。雜合作用完成後以不同濃度之 SSC 清洗尼龍膜，再以保鮮膜將尼龍膜密封，使用加強板 (Imaging plate, IP-plus III s, BAS-MP, 2040S, Fujifilm, Japan) 於室溫下進行自動放射顯影，並依據信號強弱決定曝光時間，再利用生化影像分析儀 (Bioimage-analyer system-1000, Fuji, Japan) 進行分析，並以軟體 (Kodak 1D image analysis software) 測定其訊號強度。

Streptomyces sp. RS70 處理番茄後誘發 *PR-1* mRNA 之測定

以注入滲透法將 0.1 ml 之 RS70 細菌懸浮液 (10^8 cfu/ml) 注入番茄 (株齡約一個月) 最頂端已展開之第一或第二葉片內，放置於日溫 30℃，夜溫 25℃，光照 12 小時之生長箱中，對照組以0.005% Tween 20 處理。注入處理後分別於 0 時、第 5、6、7、8、9 及 10 天採集位於接種葉之下方葉片約 0.5 g，放置於 -80℃之冷凍櫃中備用。收集之葉片分別依前述方法進行 *PR-1* mRNA 表現量測定。試驗重覆六次。

Streptomyces sp. RS70 處理不同部位對番茄植株 *PR-1* mRNA 表現之影響

將 RS70 懸浮液 (10^8 cfu/ml) 分別以上述方法注入處理於葉片內及澆灌處理 (每盆澆灌 70 ml) 於每株根部，對照組則以 0.005% Tween 20 處理。在處理後第 7 天分別採集位於處理葉下方之葉片 (葉片注入處理者) 及上位葉 (由頂葉向下採集) (澆灌處理者)，進行 *PR-1* mRNA 表現量測定。所有植株之培育及處理後之植株皆放置在日溫 30℃，夜溫 25℃，光照 12 小時之生長箱中。試驗重覆六次。

結　果

葉片處理 *Streptomyces* sp. RS70 對青枯病發病之影響

頂葉經 RS70 注入處理之番茄植株，於第7 天在根部澆灌接種青枯病菌後觀察一個月，其發病指數顯著低於頂葉經 0.005% Tween 20 處理之對照組 (表一)，尤其在青枯病菌之接種濃度爲 10^5 cfu/ml時，二者間之差異更明顯。如以青枯病菌 PS152 之 4 株無致病力突變菌株處理葉片時，則發現只有其中的 PS152E 可顯著降低發病程度 (表二)。由此等結果顯示 RS70 及 PS152E 可能誘導番茄植株之抗性而減少青枯病之發病程度。

誘導抗病所需之時間

頂葉經 *Streptomyces* sp. RS70 注入處理後，在不同天數接種青枯病菌 PS152，以瞭解誘導抗病所需之時間。結果顯示頂葉處理後第 7、8 及 9 天進行澆灌接種青枯病菌者，一個月後之發病指數均顯著低於未經 RS70 處理之對照組，與對照組相較可減少約 30.4-44.4% 之發病程度 (表三)。但 RS70 處理後第 10 天則

表一、番茄葉片處理 *Streptomyces* sp. RS70 對青枯病發病之影響

Table 1. Effect of leaf treatment with *Streptomyces* sp. RS70 on the severity of bacterial wilt of tomato inoculated by soil drenching with 10^5 or 10^6 cfu/ml of *Ralstonia solanacearum* PS152

Treatment[1]	Disease index (%)			
	7[2]	14	21	30
10^6 cfu/ml of PS152				
RS70-treated	21.00*[3]	63.00*	70.00*	70.00*
CK	35.33	65.33	76.67	83.33
10^5 cfu/ml of PS152				
RS70-treated	3.00*	29.00*	40.00*	40.00*
CK	37.33	70.00	70.00	73.33

[1] A top leaf of a tomato plant was syringe-infiltrated with *Streptomyces* sp. RS70 (10^8 cfu/ml suspended in 0.005% Tween 20) or 0.005% Tween 20 only (CK) and 7 days later the root was inoculated by drenching with 10^6 or 10^5 cfu/ml of *Ralstonia solanacearum* PS152

[2] Days after inoculation with PS152.

[3] Means with star sign in the same column between RS70-treated and CK for the same inoculum concentration are significantly different (p = 0.05) according to the t-test.

無降低青枯病發病之效果，又處理後第 7 天以前亦無減少病害之能力，且有些處理之發病指數反較對照組爲高。

Streptomyces sp. RS70 處理後番茄葉片內 *PR-1* mRNA 之表現

番茄葉片經 RS70 注入處理後，可誘發植株對青枯病產生抗性，而此抗性是否與致病過程相關基因 (pathogenesis - related genes) 之表現有著因果關係，本研究針對 *PR-1* 基因之表現加以分析。RS70 注入接種後10 天內於不同時間分析位於處理葉下方之未經處理葉片的mRNA，結果如圖一 A 及 B 所示，處理後 *PR-1* 之 mRNA 表現量隨處理天數之增加而明顯增加，直至處理後第 8 天達最高峰，其 *PR-1* 表現量約較處理後 0 時增加2.3 倍，隨後在第9 和第 10 天之 *PR-1* 表現量則略爲降低。而對照組之 *PR-1* 僅有微量之表現。

RS70 之處理可增加 *PR-1* mRNA 之表現量，而同樣可誘導抗性之 PS152E 無致病力突變菌株是否也能誘導 *PR-1* 之表現，將二者分別注入接種於番茄葉片後，於第 7 及第 8 天採集接種葉下方未經處理之葉片，分析其 *PR-1* mRNA 之表現量，結果如圖二所示，RS70 及 PS152E 均可誘導番茄葉片 *PR-1* mRNA 之表現 ，RS70 及 PS152E 注入處理後第 7 天之未處理葉，其 *PR-1* mRNA 表現量分別較經 0.005% Tween 20 處理之

表二、番茄葉片處理青枯病菌 PS152 所衍生之四株無致病力突變菌株對青枯病之影響

Table 2. Effect of leaf treatment with four avirulent mutants of *Ralstonia solanacearum* PS152 on the severity of bacterial wilt of tomato inoculated 7 days later by soil drenching with 10^5 cfu/ml of *R. solanacearum* PS152

Treatment	Disease index (%)			
	7[1]	14	21	30
PS152E	18.00c[2]	26.67c	30.00c	40.00b
PS152H	49.33b	70.00b	86.67b	80.00a
PS152K	46.67ab	64.67ab	76.67ab	93.33a
PS152R	24.67c	45.33ac	60.00a	76.67a
CK (water treated)	38.00a	57.33ab	66.67ab	73.33a

[1] Days after inoculation with PS152.

[2] Means in the same column followed by the same letter are not significantly different (p = 0.05) according to Duncan's multiple range test.

表三、番茄葉片處理 *Streptomyces* sp. RS70 後誘導系統性抗青枯病所需之時間

Table 3. Times required for induction of systemic resistance against bacterial wilt in tomato by leaf treatment with *Streptomyces* sp. RS70

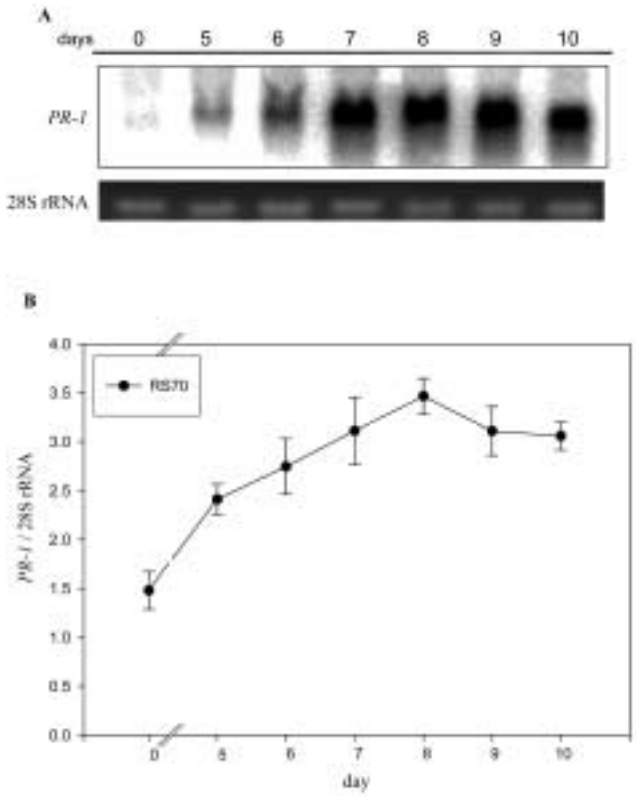
Day[1]	Treatment[2]	Disease index (%)[3]	% disease reduced[4]
1	RS70	50.00	0
	CK	50.00	
2	RS70	55.00	0
	CK	55.00	
3	RS70	75.00	0
	CK	70.00	
4	RS70	75.00	0
	CK	70.00	
5	RS70	65.00	0
	CK	45.00	
6	RS70	65.00	0
	CK	65.00	
7	RS70	53.33*	30.44
	CK	76.67	
8	RS70	50.00*	44.44
	CK	90.00	
9	RS70	47.50*	32.14
	CK	70.00	
10	RS70	36.67	0
	CK	36.67	

[1] Day of inoculation with *Ralstonia solanacearum* PS152 by soil drenching after leaf treatment with RS70.

[2] Top leaves were syringe-infiltrated with *Streptomyces* sp. RS70 (RS70) or 0.005% Tween 20 (CK).

[3] Disease recorded at 30 days after challenge-inoculation with PS152. Means with star sign between RS70-treated and CK are significantly different (P=0.05) according to t-test.

[4] Mean percent disease reduction was calculated in comparison to the control (CK).



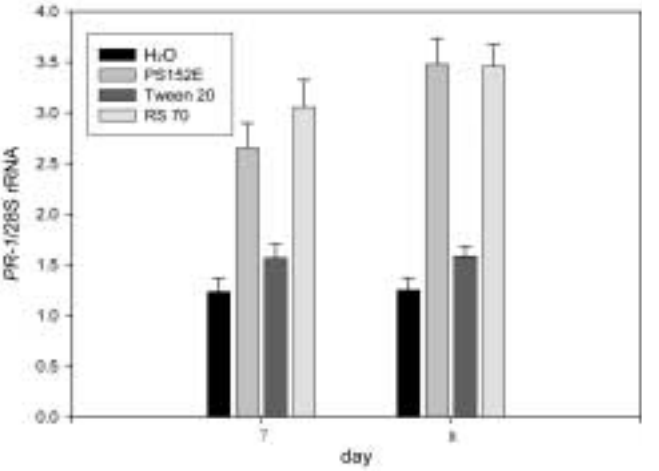
圖一、*Streptomyces* sp. RS70 注入接種於番茄葉後在下方未接種葉內所誘發 *PR-1* mRNA 之表現。

Fig. 1. Expression of *PR-1* mRNA in non-inoculated leaves of tomato after a top leaf was inoculated by syringe-infiltration with *Streptomyces* sp. RS70. Total RNA was extracted from leaves below the inoculated top leaf at each time point and analyzed by Northern hybridization. Identical blots containing 10 μg of RNA per lane were hybridized to ^{32}P-labeled *PR-1* probe (A). The intensity of the signals in blots was measured with an image analysis software (Kodak 1D image analysis software). The values of the hybridization signal obtained for *PR-1* were normalized with that of the control 28S rRNA for each time point (B). Data points are means of six replications.

對照組 (RS70 之對照組) 及無菌水處理之對照組 (PS152E 之對照組) 增加 2 及 2.1 倍，第 8 天則分別增加2.2 倍及 2.8 倍。

Streptomyces sp. RS70 處理不同部位對番茄植株 *PR-1* mRNAs 表現之影響

RS70 注入處理於葉片及澆灌處理於根部後，於第 7 天分別分析下位未處理葉片 (葉片處理者) 及上位葉 (根部處理者) 之 mRNA 時，皆能偵測到 *PR-1* mRNA 之累積 (圖三)，顯示其誘發 *PR-1* 基因表現可向上或向下移行。

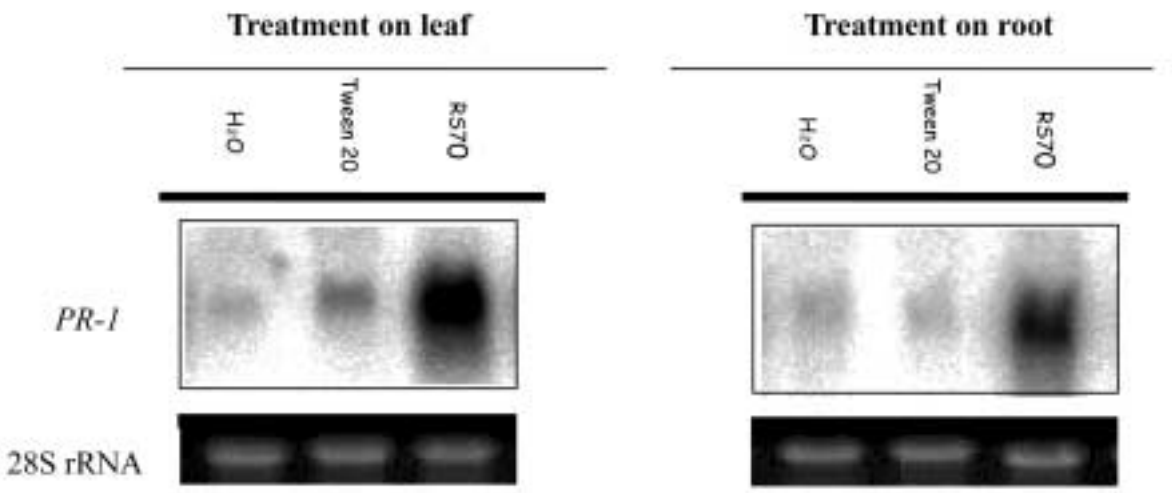


圖二、番茄葉片接種處理 *Streptomyces* sp. RS70 及青枯病菌無致病力突變菌株 PS152E 後，於第 7 天及第 8 天在下方未接種葉片內之 *PR-1* mRNA 表現量。

Fig. 2. Induction of *PR-1* expression in non-inoculated leaves of tomato after a top leaf was inoculated by syringe-infiltration with *Streptomyces* sp. RS70 or avirulent mutant PS152E of *Ralstonia solanacearum* PS152. Total RNAs were extracted from leaves below the inoculated leaves at 7 and 8 days after inoculation, and analyzed by Northern hybridization with ^{32}P-labeled *PR-1* probe. The intensity of the hybridization signals was measured with an image analysis software. The values of the signal obtained for *PR-1* were normalized with that of the control 28S rRNA. Tween 20 and H_2O were used as control in these assays for RS70 (cells were suspended in 0.005% Tween 20) and PS152E (cells were suspended in sterile distilled water), respectively.

討　論

近年來許多學者已證實非病原性之根棲細菌可誘導多種植物對植物病原眞菌、細菌及病毒產生抗性[44]，而此誘導抗性是其生物防治植物病害的機制之一。在誘導植物抗青枯病之相關研究，已有報告指出水楊酸 (salicylic acid) 澆灌土壤可誘導尤加利樹抗青枯病，而葉部處理則無法誘導抗性產生[33]；而 acibenzolar-S-methyl (Actigard) 處理番茄後則可顯著降低青枯病之發病率[2]。此外，促進植物生長之根棲細菌如 *Pseudomonas putida* 89B61 處理番茄種子後，可顯著減少青枯病之發生[2]；*P. putida* WCS358r 和 *P. fluorescens* WCS374r 處理尤加利樹葉片後，可誘發尤加利樹系統性抗青枯病[33]。本研究顯示根棲細菌 *Streptomyces* sp. RS70 防治番茄青枯病之效果[41]，也可能與誘導抗性有關連。根棲細菌能否誘導植物抗土壤傳播性病害，有些學者利用岩棉系統 (rockwool system)[21] 或分根系統



圖三、*Streptomyces* sp. RS70 以注入處理番茄葉片及以澆灌處理根部後於第 7 天對誘發未接種葉片內 *PR-1* mRNA 表現之影響。

Fig. 3. Effect of leaf and root treatments with *Streptomyces* sp. RS70 on the induction of *PR-1* expression in non-inoculated leaves of tomato. RS70 cells suspended in 0.005 % Tween 20 were either syringe-infiltrated into top leaves or drenched onto roots of potted plants. Total RNAs were extracted from leaves below inoculated leaves (for leaf treatment) or from top leaves (for root treatment) 7 days after treatment, and analyzed by Northern hybridization with ^{32}P-labeled *PR-1* probe. H_2O and Tween 20 represent treatments with sterile distilled water and 0.005% Tween 20 only, respectively.

(split root system)[5] 之設計來加以證明，因這些設計可有效地將誘導細菌與病菌作空間隔離，而便於排除因接觸而發生之競爭及抗生作用。由於番茄幼苗之根細小，不易分根處理，而岩棉系統需有特殊材料，故本研究以不同部位之接種方式，亦即將誘導細菌與病菌分別接種於葉及根部來探討誘導抗性之發生。當 *Streptomyces* sp. RS70 先接種番茄頂葉後第 7 天，再將青枯病菌接種於根部時，青枯病之發病程度顯著降低，此表示 RS70 可能誘導番茄系統性抗青枯病。

植物需要時間才能達到誘導階段，所以施用誘導原 (inducer) 並不能在植物保護上達到立即性效果[44]，許多研究顯示其通常需要數天至數週來發展 SAR 或 ISR[13]，而此時間依植物及誘導生物種類之不同而異，例如，*P. fluorescens* WCS374 誘導蘿蔔抗 *Fusasrium* 萎凋病，至少需要一天的時間才能誘發出顯著抗性[21]；*P. putida* WCS358r 和 *P. fluorescens* WCS374r 需要 3-5 天的時間才能誘導尤加利樹抗細菌性萎凋病[33]；而阿拉伯芥 (Arabidopsis) 則需暴露在 *Bacillus subtilis* GB03、*Bacillus amyloliquefaciens* IN937a 之揮發性有機化合物 (volatile organic compounds) 中 4-14 天，才足以活化誘導阿拉伯芥抗細菌性軟腐病[35]。此外，胡瓜在接種 *Pseudomonas syringae* 7 小時後即能產生 SAR[36]。本研究發現番茄葉部經 *Streptomyces* sp. RS70 注入處理後，需要至少六天才能誘導番茄對青枯病之抗性，且此抗性僅維持數天，因此，如何延續其抗性，在防治之應用至為重要。Kuć 及Richmond (1977)[20] 報告胡瓜葉片在初次接種 *Collectotrichum lagenarium* 3 週後進行第二次輔助接種，其誘導保護效力可持續至果實期，又 Anith 等人[2] 發現 *Pseudomonas putida* 89B61 包覆處理番茄種子後，並不能顯著地降低青枯病之發病率，但在幼苗接種青枯病菌前 7 天再作第二次輔助接種，則可顯著地降低青枯病之發病率，由此可知有些情況下施以輔助接種，可延長或提升其誘導抗性。在番茄上，RS70 之輔助接種處理能否延長抗病性，有待進一步研究。

PGPR 誘導之 ISR 通常與 PR 蛋白質之累積並無直接關聯性[14, 15, 30, 47]，但本研究發現 RS70 處理可誘發番茄 *PR-1* 基因之表現，此結果與 Maurhofer[27] 等人之研究結果相似，他們曾報告 *P. fluorescens* strain CHAO 誘導菸草對菸草壞疽病毒 (tobacco necrosis virus) 產生之抗性與 PR 蛋白質之累積有著關連性。RS70 處理後所誘發番茄 *PR-1* mRNA 之累積量，隨時間而增加，至第 8 天達最高量，且在第 7 至第 10 天之間其量均高。前已提及 RS70 處理後需至第 7 天才能表現顯著的抗性，且在第 8 及第 9 天仍維持其抗性，因此抗性之表現似與 *PR-1* 之累積量有相關，但在第十天雖有高量之 *PR-1* mRNA 累積，卻無抗性之表現，其原因不明，因本研究僅測定至第十天為止。*PR-1* 之量是否在第十天後即迅速下降，以致抗性消失有待探討。在 SAR 的發展過程中會產生一訊號，其在植物體內從誘導位置可向

上和向下運送，以致遠距離組織亦能產生誘導狀態[4, 13, 34, 37]；而根棲細菌所啓動之 ISR 亦能相似地產生移行性訊號以誘導保護根和枝幹[44]。本研究及先前試驗結果顯示不論是番茄葉片注入或根部澆灌 RS70 後，皆可降低青枯病之發病程度[41]，而本研究之北方雜合分析顯示不論是番茄葉片注入或根部澆灌處理 RS70 後，於未處理之葉片皆能偵測到 *PR-1* 基因的表現，此結果表示誘發 *PR-1* 基因表現之訊號可雙向移行，亦即可向上或向下移行，也可能說明不論是植株葉部處理或根部澆灌處理皆能降低病害發生之效果。

傳統上藉由弱病原菌可誘導產生 SAR 對眞菌、細菌或病毒產生抗性[4, 38]，本研究也探討青枯病菌無致病力之四株突變株能否誘導抗性，結果顯示其中一株能誘導番茄對青枯病之抗性，且此突變株亦可誘發番茄 *PR-1* 基因之表現，故與 RS70 誘導抗性之現象相似。葉片在注入接種此突變株後會形成局部壞疽病斑，而壞疽病斑之產生已知是病原菌誘發 SAR 所必需[38]。無致病力突變株與 RS70 均可誘導番茄產生抗性，但在應用上 RS70 較爲適宜，因其爲腐生菌，又可促進植物生長，且施用於種子或栽培介質中均有防治青枯病之效果[41]。

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ABSTRACT

Teng, Y. C[1]., Tzeng, K. C[1]., and Hsu, S. T[1,2]. 2006. Induction of systemic resistance in tomato against bacterial wilt by a plant growth-promoting rhizobacterium *Streptomyces* sp. RS70. Plant Pathol. Bull. 15:107-116 ([1]Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan ; [2]Corresponding author, Email : sthsu@mail.nchu.edu.tw ; Fax : +886-4-22877585)

Streptomyces sp. RS70 is a rhizobacterium capable of promoting tomato growth and reducing bacterial wilt disease caused by *Ralstonia solanacearum* following either seed coating or seedling drenching. However, it has no inhibitory activity against *R. solanacearum* in vitro. To understand the mechanism by which *Streptomyces* sp. RS70 controls the bacterial wilt, we investigated whether RS70 could induce systemic resistance in tomato. The severity of bacterial wilt was significantly reduced when the top leaves of tomato seedlings were syringe-infiltrated with *Streptomyces* sp. RS70 and challenge-inoculated by root drenching with strain PS152 of *R. solanacearum* 7 days post infiltration, indicating that RS70 may have induced systemic disease resistance. At least six days after leaf infiltration with RS70 were required for effective resistance to be induced. However, the induced resistance persisted only for several days. The Northern blot analysis revealed that RS70 treatment could induce *PR-1* gene expression in noninoculated leaves, and in a 10-day test period, the quantity of *PR-1* mRNA increased with time, reaching the highest at the 8th day and then slightly decreased. Accumulation of *PR-1* mRNA in noninoculated leaves was observed not only by leaf infiltration but also by root treatment with RS70, indicating that the signal for *PR-1* gene expression induced by RS70 may be transported downward and upward from the inoculated site. Similar to RS70, one of the four tested avirulent mutants of *R. solanacearum* PS152, was able to induce systemic resistance and increase *PR-1* gene expression.

Key Word：rhizobacteria, induced resistance, bacteria wilt disease, tomato, *PR-1* gene