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產氰綠膿桿菌對植物病原細菌生長之影響

林宜賢[1]　張世杰[1]　蔡沟龍[1]　徐世典[1]　黃秀珍[2]　曾國欽[1, 3]

1 國立中興大學植物病理學系
2 國立中興大學生物科技學研究所
3 聯絡作者，電子郵件：kctzeng@nchu.edu.tw ；傳眞：+886-4-22854633
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摘　要

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由葉表分離之綠膿桿菌 (*Pseudomonas aeruginosa*) 常具有產生氰酸的特性，而螢光假單胞菌氰酸的產生爲防治植物眞菌性病害的機制之一。本研究利用密閉培養皿系統測試葉表分離之綠膿桿菌菌株對不同植物病原細菌在培養基上之生長，發現產氰能力強之菌株，除對*Erwinia chrysanthemi* CAS7 外，可完全抑制 *Agrobacterium tumefaciens* RS3 、*Erwinia carotovora* subsp. *carotovora* ZL1 、*Pseudomonas syringae* PA5 、*Ralstonia solanacearum* PS152 及 *Xanthomonas axonopodis* pv. *vesicatoria* XVT40 之生長，且綠膿桿菌之產氰能力與抑菌程度具正相關。利用不產氰之 *P. putida* YLFP44 菌株，經轉形所得之產氰衍生菌株，來探討氰酸在抑菌作用之角色，結果顯示產氰衍生菌株 Y44H1 (帶有由 *P. aeruginosa* WFP11r 選殖之 *hcnABC* 基因)，除對 *Erwinia chrysanthemi* CAS7 外，亦可完全抑制其他供試之植物病原細菌的生長，及完全抑制不同寄主來源的青枯病菌，但僅帶有選殖載體的衍生菌株 Y44C1，則不能產生氰酸亦無抑菌之能力，表示產氰菌株之抑菌作用與其氰酸的產生有關。Y44H1 衍生菌株對青枯病菌不僅抑制其生長，處理時間長時(5 天後) 也有致死作用，此外並可造成菌落及細胞形態之改變。

關鍵詞：綠膿桿菌、氰酸、植物病原細菌、青枯病菌、抑制生長

緒　言

由植物葉表所分離到的許多綠膿桿菌 *Pseudomonas aeruginosa* (Schroeter) Migula 菌株具有產生氰酸(簡稱產氰)的能力 [1]。綠膿桿菌曾被應用於植物病害的生物防治，如 *P. aeruginosa* 7NSK2 菌株可降低由 *Pythium* spp. 所引起之番茄猝倒病 [6] 及誘導菜豆對 *Botrytis cinerea* Pers. ex Fr. 所引起之灰黴病產生系統性抗病 [8, 9] ；產氰之 *P. aeruginosa* IE-6$^+$ 菌株可應用於防治由 *Fusarium* spp.、*Rhizoctonia solani* Kuhn 及 *Pythium* spp. 所引起之番茄病害 [18]。具拮抗作用之螢光假單胞菌，包含 *P. putida* (Trevisan) Migula 及 *P. fluorescens* (Trevisan) Migula，可降低青枯病的發生 [5] ；趙氏 [4] 由番茄根圈所分離之螢光假單胞菌菌株中，有些菌株亦具有防治番茄青枯病的潛力，然部分菌株則反而有促進病害發生的現象。螢光假單胞菌氰酸的產生爲防治眞菌性病害的機制之一 [10, 21]，至於其在植物細菌性病害的生物防治上所扮演的角色仍未有報導。本研究測定綠膿桿菌所產生的氰酸對 *Agrobacterium tumefaciens* (Smith and Townsend) Conn RS3 (玫瑰癌腫病菌)、*Erwinia carotovora* subsp. *carotovora* (Jones) Bergey ZL1 (彩色海芋細菌性軟腐病菌)、*Erwinia chrysanthemi* Burkholder *et al*. CAS7 (青蔥細菌性軟腐病菌)、*Pseudomonas syringae* van Hall PA5 (楊桃細菌性斑點病菌)、*Ralstonia solanacearum* (Smith) Yabuuchi *et al*. PS152 (番茄青枯病菌) 及 *Xanthomonas axonopodis* pv. *vesicatoria* Vauterin *et al*. XVT40 (番茄細菌性斑點病菌) 等植物病原細菌生長的抑制能力，並以 *P. aeruginosa* WFP11r 菌株基因體中所選殖的產氰基因，用電轉形法送入不產氰的 *P. putida* YLFP44 後所獲得產氰之衍生菌株 Y44H1 [17]，探討氰酸在抑菌作用所扮演之角色及對青枯病菌之生長、菌落及細胞形態的影響。

材料與方法

供試菌株及培養

本研究中所使用之植物病原細菌、綠膿桿菌及其他螢光假單胞菌各菌株，皆爲國立中興大學植物病理學系植物病原細菌研究室所保存之菌株，綠膿桿菌及其他螢光假單胞菌菌株之來源及其氰酸產生特性列於表一。試驗進行前，將 *Agrobacterium*、*Erwinia* 及 *Xanthomonas* 屬細菌培養於 nutrient agar (NA; Difco nutrient agar powder, 23g; distilled water, 1L; pH7.2)、*Ralstonia solanacearum* 培養於 tetrazolium chloride (TZC) 培養基[13] 及 *Pseudomonas* 屬細菌則培養於 King's medium B (KB) [14] 培養基上，於 30℃ 培養後進行各項試驗。而含有 *P. aeruginosa* WFP11r 之 *hcnABC* 基因之 *P. putida* YLFP44 衍生菌株 Y44H1 與僅含有選殖載體 pBBR1MCS-5 之衍生菌株 Y44C1 [2, 17] 則培養於含有 20 μg/ ml gentamycin 之KB 培養基(KBG)。

產氰綠膿桿菌及 *Pseudomonas putida* YLFP44 衍生菌株對植物病原細菌生長之影響

爲探討產氰綠膿桿菌氰酸的產生對植物病原細菌生長的抑制能力，本試驗以密閉培養皿系統進行測試。將培養於 KB 或 KBG 培養基上 16 小時之各綠膿桿菌菌株或 *P. putida* YLFP44 之衍生菌株 Y44H1 或 Y44C1 以無菌蒸餾水懸浮，製備成 OD_{620} 爲 0.3 之細菌懸浮液(細菌濃度約爲 10^8 CFU/ ml) 後，經 10 倍稀釋成 10^7 CFU/ ml 之接種源，另將新鮮培養之各植物病原細菌菌株懸浮於無菌蒸餾水中，製備成 OD_{620} 爲 0.3 之細菌懸浮液，並系列稀釋成 10^4 CFU/ ml 作爲接種源。利用全自動迴旋式細菌塗佈儀 (Whitley automatic spiral plater, Don Whitley Scientific Limited, England) 將綠膿桿菌或螢光假單胞菌衍生菌株之懸浮液，分別塗佈於直徑 9 cm 的 KB 或 KBG 培養基平板上，同時也將植物病原細菌懸浮液，分別塗抹於另一直徑相同之 NA、TZC 或 KB 培養基平板上。隨後將塗抹綠膿桿菌或螢光假單胞菌衍生菌株之培養皿底部平板，分別與塗抹植物病原細菌之培養皿底部平板，面對面相疊並以石蠟膜(Parafilm, WI, USA) 密封，共同培養於 30℃，經 48 小時觀察植物病原細菌各菌株的生長，依據 Gilchrist *et al.* [11] 之方法計數特定區域內之菌落數目，並回推塗佈細菌之存活數目，單位以 log (CFU+1)/ plate 表示。每一處理皆爲三重覆。

Pseudomonas putida YLFP44 產氰衍生菌株Y44H1 對青枯病菌不同菌株之影響及殺菌能力之測試

爲測試氰酸對不同作物所分離之青枯病菌菌株生長之抑制作用是否有差異，本研究以螢光假單胞菌 *P. putida* YLFP44 衍生菌株 Y44H1 爲產氰之供試菌株，此 Y44H1 是由不產氰 *P. putida* YLFP44 菌株經電轉形獲得由 *P. aeruginosa* WFP11r 選殖之 *hcnABC* 基因後的衍生菌株，另以僅轉入選殖載體 pBBR1MCS-5，而不產氰的 Y44C1 衍生菌株作爲對照菌株 (表一)。依前述之方法分別測試此二衍生菌株於密閉培養皿系統中對由番茄、馬鈴薯、甜椒及長茄所分離之青枯病菌菌株在生長上之影響；此外於 Y44H1 之處理組中，分別在培養 3 天、5 天及 7 天後，依前述 Gilchrist *et al.* [11]之方法，計數青枯病菌 PS152 菌株固定區域內的菌落數目，並將石蠟膜解開移除培養 Y44H1 之平板，隨即將培養青枯病菌 PS152 菌株之平板，蓋上培養皿頂蓋繼續培養 2 天，再計數固定區域內之菌落數目，以測定 Y44H1 菌株所產生之氰酸對 PS152 菌株是抑制或是殺菌作用。依所計數之菌落數推算塗佈細菌之存活數目 [11]，單位以 log (CFU+1)/ plate 表示。每一處理爲三重複。

穿透式電子顯微鏡之觀察

爲進一步探討 *P. putida* YLFP44 衍生菌株 Y44H1 氰酸

表一、本研究所使用之植物來源螢光假單胞菌菌株及其產生氰酸之能力

Table 1. Fluorescent pseudomonad strains from plants used in this study

Strain	Plant origin or genotype	Cyanide [1]	Reference
Pseudomonas aeruginosa			
WFP11r	Chrysanthemum	+ +	(1)
WFP171	Common Reedgrass	+ +	(17)
WFP36	Chrysanthemum	+	(1)
WFP23	Bird of paradise	-	(1)
WFP135r	Carnation	-	(1)
Pseudomonas putida			
YLFP44	Sweet pepper	-	(20)
Y44H1	YLFP44 harboring pBBR1MCS-5::3.1kb *hcnABC* genes; Gm^r	+ +	(17)
Y44C1	YLFP44 harboring pBBR1MCS-5; Gm^r	-	(17)

[1] The degree of hydrogen cyanide production: + +, strong; +, weak; -, none.

產生對青枯病菌細胞形態之影響，本試驗先將青枯病菌 PS152 菌株培養於 TZC 培養基上 2 天，使菌落產生，再依上述密閉培養皿系統分別以 Y44H1 及 Y44C1 處理 5 天後，觀察青枯病菌 PS152 菌株之菌落形態，並以無菌蒸餾水懸浮平板上之細菌，製備爲 OD_{620} 爲 0.3 之細菌懸浮液。將所製備之細菌懸浮液利用陰染法 (negative stain) [3] 分別經 0.1% bacitracin (Sigma Chemical Co., MO, USA) 與 2% 醋酸鈾 (Uranyl acetate, Sigma Chemical Co., MO, USA) 染色，再利用穿透式電子顯微鏡 (JEM-1200CX II, JEOL, Tokyo, Japan) 觀察菌體之形態。

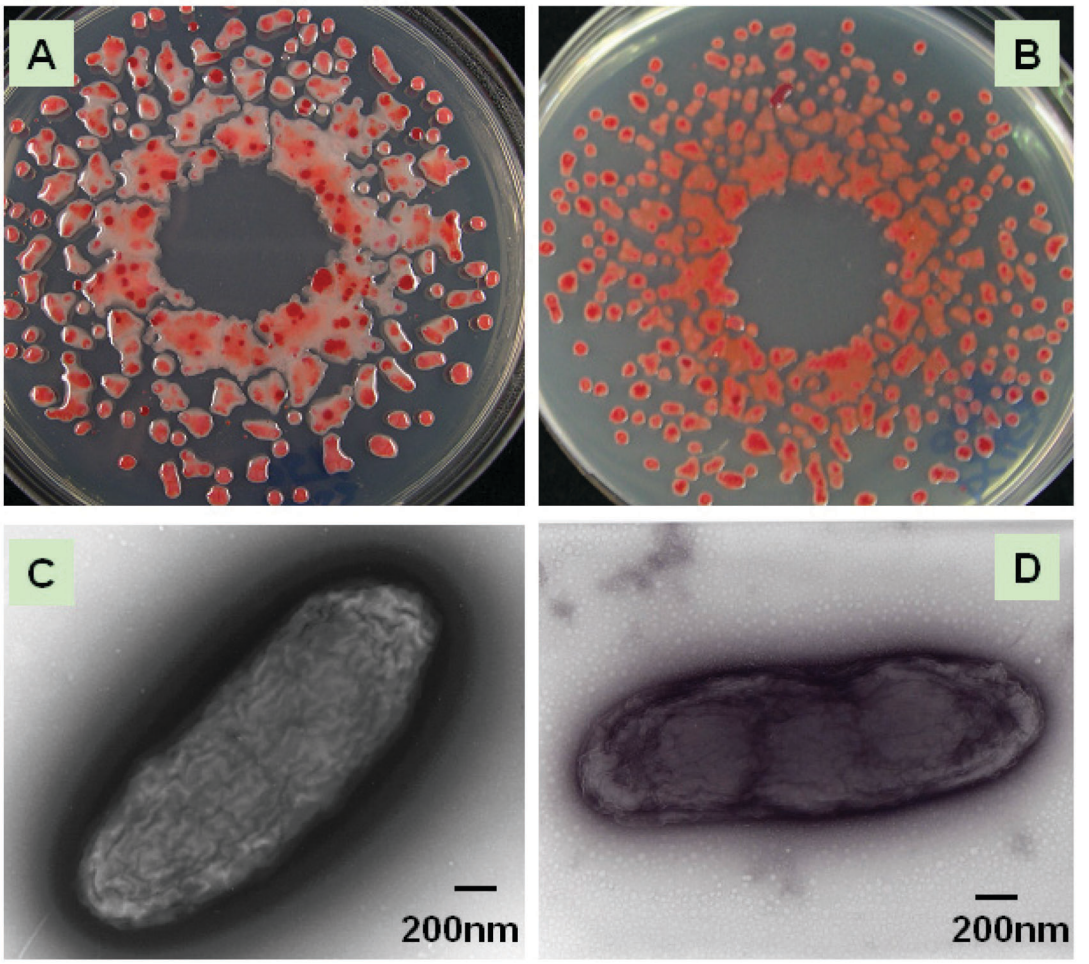
結　果

產氰綠膿桿菌對植物病原細菌生長之影響

在密閉培養皿系統之測試結果，顯示產氰能力較強之 *P. aeruginosa* WFP11r 與 WFP171 菌株，除對 *E. chrysanthemi* CAS7 菌株外，皆可完全抑制其他五種供試之植物病原細菌菌株之生長 (表二)；氰酸產生能力較低之 WFP36 菌株，除可完全抑制 *X. axonopodis* pv. *vesicatoria* XVT40 菌株外，對其餘植物病原細菌菌株生長的抑制能力弱或無抑制能力，而不產氰之 *P. aeruginosa* WFP23 與 WFP135r 菌株，則對所有供試植物病原細菌菌株之生長皆沒有抑制作用 (表二)。

Pseudomonas putida YLFP44 衍生菌株對植物病原細菌生長之影響

在此密閉培養皿系統中，產氰之 *P. putida* YLFP44 衍生菌株 Y44H1，除對 *E. chrysanthemi* CAS7 菌株外，亦可完全抑制其他五種供試之植物病原細菌菌株之生長 (表三)，而不產氰之 *P. putida* YLFP44 衍生菌株 Y44C1 則對所有供試植物病原細菌菌株之生長沒有抑制作用 (表三)。



圖一、*Ralstonia solanacearum* PS152 菌株在 TZC 培養基上生長二天後置於密閉培養皿系統中以 *Pseudomonas putida* 不產氰衍生菌株 Y44C1 (A, C) 或產氰衍生菌株 Y44H1 (B, D) 處理五天所形成之菌落及細胞形態

Fig. 1. The colony type on tetrazolium chloride (TZC) medium and the cell morphology of *Ralstonia solanacearum* PS152 five days after treatment (co-incubation) with *Pseudomonas putida* YLFP44 noncyanogenic derivative Y44C1 (A, C) or cyanogenic derivative Y44H1 (B, D) in a closed paired-plate system. *Ralstonia solanacearum* PS152 was grown on TZC medium for 2 days before treatment with Y44C1 or Y44H1.

表二、產氰綠膿桿菌在密閉培養皿系統中對不同植物病原細菌菌株生長之影響

Table 2. Effect of cyanogenic *Pseudomonas aeruginosa* strains on the growth of plant pathogenic bacteria incubated in a closed paired-plate system

	Growth of plant pathogenic bacterium after incubation with					
	WFP11r	WFP171	WFP36	WFP23	WFP135r	Control [4]
Plant pathogenic bacterium	++ [3]	++	+	-	-	-
Agrobacterium tumefaciens RS3	0.0 b [2]	0.0 b	3.4 a	3.4 a	3.4 a	3.4 a
Erwinia carotovora subsp. *carotovora* ZL1	0.0 c	0.0 c	2.7 b	3.4 a	3.4 a	3.3 a
Erwinia chrysanthemi CAS7	3.4 a	3.4 a	3.4 a	3.3 a	3.4 a	3.4 a
Pseudomonas syringae PA5	0.0 b	0.0 b	3.3 a	3.3 a	3.4 a	3.4 a
Ralstonia solanacearum PS152	0.0 c	0.0 c	1.9 b	2.7 a	2.7 a	2.7 a
Xanthomonas axonopodis pv. *vesicatoria* XVT40	0.0 b	0.0 b	0.0 b	2.7 a	2.7 a	2.7 a

1. An automatic spiral plater system (Don Whitley Scientific Limited, England) was used to spread cell suspension of each bacterial strain tested onto agar plate. One nutrient agar (NA) or tetrazolium chloride (TZC) plate [13] spread with a 50 μl of cell suspension (10^4 CFU/ ml) of each plant pathogenic bacterial strain was paired with one King's medium B (KB) plate spread with a 50 μl of cell suspension (10^7 CFU/ ml) of each *P. aeruginosa* strain, respectively. The paired plates assembly were then sealed with parafilm. The colonies formed by *R. solanacearum* PS152 on TZC plate and by other bacterial strains tested on NA plates 48 hr after incubation at 30℃ were examined and calculated according to the method described by Gilchrist *et al*. [11].
2. Each value is the mean of three replicates presented in log (CFU+1)/ plate and values in the same row followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.
3. Symbols indicate the degree of hydrogen cyanide production by the strain of *P. aeruginosa* as footnote in Table 1.
4. Plate spread with cell suspension of each plant pathogenic bacterial strain was paired with blank KB plate on which no cell suspension of bacteria was spread.

表三、*Pseudomonas putida* YLFP44 產氰衍生菌株 Y44H1 與不產氰衍生菌株 Y44C1 在密閉培養皿系統中對不同植物病原細菌菌株生長之影響

Table 3. Effect of *Pseudomonas putida* YLFP44 cyanogenic derivative Y44H1 and noncyanogenic derivative Y44C1 on the growth of plant pathogenic bacteria incubated in a closed paired-plate system

	Growth of plant pathogenic bacterium after incubation with		
	Y44H1	Y44C1	Control [4]
Plant pathogenic bacterium [1]	++ [3]	-	-
Agrobacterium tumefaciens RS3	0.0 b [2]	2.6 a	2.6 a
Erwinia carotovora subsp. *carotovora* ZL1	0.0 b	2.2 a	2.3 a
Erwinia chrysanthemi CAS7	2.4 a	2.4 a	2.4 a
Pseudomonas syringae PA5	0.0 b	2.6 a	2.6 a
Ralstonia solanacearum PS152	0.0 b	2.6 a	2.7 a
Xanthomonas axonopodis pv. *vesicatoria* XVT40	0.0 b	2.6 a	2.7 a

1. The cell suspension of each bacterial strain tested was spread onto agar plate as footnote in Table 2. One nutrient agar (NA) or tetrazolium chloride (TZC) plate [13] spread with a 50 μl of cell suspension (10^4 CFU/ ml) of each plant pathogenic bacterial strain was paired with one King's medium B supplemented with gentamycin 20 μg/ ml (KBG) plate spread with a 50 μl of cell suspension (10^7 CFU/ ml) of each *P. putida* derivative, respectively. The paired plates assembly were then sealed with parafilm. The colonies formed by *R. solanacearum* PS152 on TZC plate and by other bacterial strains tested on NA plates 48 hr after incubation at 30℃ were examined and calculated according to the method described by Gilchrist *et al*. [11].
2. Each value is the mean of three replicates presented in log (CFU+1)/ plate and values in the same row followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.
3. Symbols indicate the degree of hydrogen cyanide production by the derivatives of *P. putida* as footnote in Table 1.
4. Plate spread with cell suspension of each plant pathogenic bacterial strain was paired with blank KBG plate on which no cell suspension of bacteria was spread.

表四、螢光假單胞菌 *Pseudomonas putida* YLFP44 之產氰衍生菌株 Y44H1 及不產氰衍生菌株 Y44C1 在密閉培養皿系統中對不同寄主來源青枯病菌菌株生長之影響

Table 4. Effect of *Pseudomonas putida* YLFP44 cyanogenic derivative Y44H1 and noncyanogenic derivative Y44C1 on the growth of *Ralstonia solanacearum* strains from different host incubated in a closed paired-plate system

R. solanacearum strain [1]	Growth of *R. solanacearum* strain after incubation with		
	Y44H1	Y44C1	Control [3]
Tomato			
PS21	0.0 b [2]	3.2 a	3.0 a
PS95	0.0 b	3.3 a	3.0 a
PS152	0.0 b	3.1 a	3.0 a
Potato			
PS60	0.0 b	3.2 a	3.0 a
PS92	0.0 b	3.3 a	3.1 a
Po1	0.0 b	3.4 a	3.0 a
Sweet pepper			
PS91	0.0 b	3.1 a	3.0 a
PS96	0.0 b	3.2 a	3.0 a
Eggplant			
PS99	0.0 b	3.1 a	3.1 a
PS100	0.0 b	3.1 a	3.1 a

1. The cell suspension of each bacterial strain tested was spread onto agar plate as footnote in Table 2. One tetrazolium chloride (TZC) plate [13] spread with a 50 μl of cell suspension (10^4 CFU/ ml) of each *R. solanacearum* strain was paired with one King's medium B supplemented with gentamycin 20 μg/ ml (KBG) plate spread with a 50 μl of cell suspension (10^7 CFU/ ml) of each *P. putida* derivative, respectively. The paired plates assembly were then sealed with parafilm. The colonies formed on TZC plate by each *R. solanacearum* strain 48 hr after incubation at 30℃ were examined and calculated according to the method described by Gilchrist *et al.* [11].
2. Each value is the mean of three replicates presented in log (CFU+1)/ plate and values in the same row followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.
3. Plate spread with cell suspension of each *R. solanacearum* strain was paired with blank KBG plate on which no cell suspension of bacteria was spread.

P. putida YLFP44 產氰衍生菌株Y44H1 對青枯病菌不同菌株之影響及殺菌能力之測試

產氰之 *P. putida* 衍生菌株 Y44H1 在密閉培養皿系統中，可完全抑制由不同茄科作物所分離之青枯病菌菌株之生長，但不產氰之衍生菌株 Y44C1 則無抑制能力(表四)。青枯病菌 PS152 菌株與 Y44H1 菌株於密閉培養皿系統中共同培養處理 3 天，將 Y44H1 菌株之平板移除後，PS152 菌株仍可於 48 小時後有菌落的產生，且菌落數與無處理 Y44H1 之對照組相同，然與 Y44H1 菌株共同培養處理 5 天後，則無菌落之產生(表五)。青枯病菌 PS152 菌株先培養於 TZC 培養基上 2 天，使菌落產生後再分別以衍生菌株 Y44H1 或 Y44C1 處理 5 天，結果發現以 Y44H1 處理時，青枯病菌之菌落變爲較乾燥、較少流質黏液之特性，而以 Y44C1 處理之菌落則呈流質黏液狀(圖一、A, B)。以電子顯微鏡觀察發現以 Y44H1 處理過之青枯病菌 PS152 菌株細胞(達 90% 以上)，菌體呈現皺縮不平整之現象(圖一、C, D)。

表五、*Ralstonia solanacearum* PS152 菌株於密閉培養皿系統中與 *Pseudomonas putida* YLFP44 產氰衍生菌株 Y44H1 共同培養不同天數，及移除Y44H1 後48 小時之生長

Table 5. Growth of *Ralstonia solanacearum* PS152 incubated for different days with or without *Pseudomonas putida* YLFP44 cyanogenic derivative Y44H1 in a closed paired-plate system, and 48 hr after removal of Y44H1

Treatment [1]	Growth of *R. solanacearum* PS152		
	3 day	5 day	7 day
In paired-plate system			
With Y44H1	0.0 b [2]	0.0 b	0.0 b
Without Y44H1	3.3 a	3.1 a	3.2 a
After removal of Y44H1	3.3 a	0.0 b	0.0 b

1. The cell suspension of each bacterial strain tested was spread onto agar plate as footnote in Table 2. One tetrazolium chloride (TZC) plate [13] spread with a 50 μl of cell suspension (10^4 CFU/ ml) of *R. solanacearum* PS152 was paired with one King's medium B supplemented with gentamycin 20 μg/ ml (KBG) plate spread with a 50 μl of cell suspension (10^7 CFU/ ml) of each *P. putida* derivative, respectively. The paired plates assembly were then sealed with parafilm and incubated for 3, 5 or 7 days at 30℃. Afterward KBG plate spread with Y44H1 was removed from the paired-plate system and substituted with one blank KBG plate on which no cell suspension of bacteria was spread. The colonies formed on TZC plate by *R. solanacearum* PS152 on 3, 5 or 7 days after incubation at 30℃ and 48hr after removal of Y44H1 were examined and calculated according to the method described by Gilchrist *et al.* [11].
2. Each value is the mean of three replicates presented in log (CFU+1)/ plate and values in the same column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

討　論

綠膿桿菌常存在於自然環境中，葉表綠膿桿菌常具有產氰之能力 [1]，本研究結果顯示產氰能力強之綠膿桿菌菌株，如 WFP11r 與 WFP171 菌株於密閉培養皿系統中，對多種植物病原細菌之生長有完全抑制之能力，此抑制能力與綠膿桿菌氰酸產生能力有正相關。此外，本研究利用獲得綠膿桿菌產氰基因 *hcnABC* 之 *P. putida* YLFP44 產氰衍生菌株 Y44H1，在密閉培養皿系統中，對植物病原細菌之抑菌能力與產氰綠膿桿菌對供試植物病原細菌的抑菌結果相似，而僅轉入不帶有 *hcnABC* 基因的選殖載體後，所得

到的不產氰衍生菌株 Y44C1，對所有供試植物病原細菌之生長，則無抑制作用。此結果進一步證明氰酸確爲產氰綠膿桿菌對植物病原細菌之抑菌因子。在測試的植物病原細菌中，*E. chrysanthemi* CAS7 菌株之生長不受產氰綠膿桿菌所抑制，由於 *E. chrysanthemi* 菌株對 KCN 之忍受性具有差異性[15]，其他寄主來源之 *E. chrysanthemi* 菌株之生長，是否亦會受產氰綠膿桿菌所抑制則有待測試。部分細菌如綠膿桿菌、*Bacillus pumilus*、*P. putida* O1G3 具有可分解或忍受氰酸的酵素[7, 12, 19]，而 *E. chrysanthemi* CAS7 菌株爲何對氰酸具有抗性，其機制亦值得探討。在密閉培養皿系統中，Y44H1 可完全抑制青枯病菌不同寄主來源菌株之生長，進一步將青枯病菌 PS152 菌株於密閉培養皿系統中以產氰之 Y44H1 分別處理 3, 5, 7 天，青枯病菌 PS152 菌株於 TZC 培養基上皆無法形成菌落；然以 Y44H1 處理 3 天，將 Y44H1 移除後，經 48 hr 培養則可見青枯病菌 PS152 菌株菌落長出，可見以 Y44H1 處理 3 天者，對青枯病菌係屬靜菌作用；而經 Y44H1 處理 5 或 7 天者，在移除 Y44H1 後，則青枯病菌已無法再形成菌落，其對青枯病菌應屬殺菌作用，推測可能因處理時間較長，氰酸已累積到達殺菌之濃度所致。此外青枯病菌經培養產生菌落後，再以產氰之 Y44H1 菌株處理 5 天，發現菌落形態會失去原有之流質黏液狀，於電子顯微鏡下觀察發現青枯病菌菌體已呈現皺縮不平整之現象，而氰酸對於植物病原細菌細胞內部顯微構造之影響，則有待進一步探討。雖然產氰綠膿桿菌在密閉培養皿系統中對多種植物病原細菌具有抑制能力，其與植物病原細菌於自然生態環境中之交互關係，則有待進一步測試，而 Lin *et al.*[16, 17] 之研究則顯示，產氰能力較強之綠膿桿菌菌株，對蔬菜作物之發芽與幼苗之生長有抑制作用，且可能促進番茄青枯病之發生。

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ABSTRACT

Lin, Y. H.[1], Chang, S. C.[1], Tsai, Y. L.[1], Hsu, S. T.[1], Huang, H. C.[2], and Tzeng, K. C.[1, 3] 2004. Effect of cyanogenic *Pseudomonas aeruginosa* on the growth of plant pathogenic bacteria. Plant Pathol. Bull. 13:211-218. ([1.] Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan; [2.] Graduate Institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan; [3.] Corresponding author, E-mail: kctzeng@nchu.edu.tw; Fax: +886-4-22854633)

Many strains of *Pseudomonas aeruginosa* from plants are cyanogenic, and the production of hydrogen cyanide (HCN) is one of the factors involved in the control of certain fungal diseases. The effect of cyanogenic *Pseudomonas aeruginosa* strains on the growth of plant pathogenic bacteria was studied in a closed paired-plate system. Strong cyanogenic strains completely inhibited the growth of *Agrobacterium tumefaciens* RS3, *Erwinia carotovora* subsp. *carotovora* ZL1, *Pseudomonas syringae* PA5, *Ralstonia solanacearum* PS152 and *Xanthomonas axonopodis* pv. *vesicatoria* XVT40, but not *Erwinia chrysanthemi* CAS7. The inhibitory activity was correlated with the HCN production ability of *P. aeruginosa* strains. The role of HCN in this growth inhibition was examined by testing the inhibitory ability of a cyanogenic transformant derived from noncyanogenic strain YLFP44 of *P. putida*. The results showed that *P. putida* derivative Y44H1 (harboring the cloned *hcnABC* genes from *P. aeruginosa* WFP11r) capable of producing HCN was able to inhibit the growth of different strains of *R. solanacearum*, whereas derivative Y44C1 (noncyanogenic, harboring only the cloning vector) was not, indicating the growth inhibition by the cyanogenic strain was related to the production of HCN. Prolonged treatment (5 days) with Y44H1 caused death of *R. solanacearum* and also altered the colony type and cell morphology of *R. solanacearum*.

Key words : *Pseudomonas aeruginosa*, hydrogen cyanide, plant pathogenic bacteria, *Ralstonia solanacearum*, growth inhibition