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碳氮源影響拮抗細菌防治百合灰黴病的效應

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摘　要

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評估碳素源及氮素源對 *Bacillus cereus* D-NYDA-602 和 *Paenibacillus polymyxa* Y-NYDA-21 兩株拮抗細菌抑制百合灰黴病 (由 *Botrytis elliptica* 引起) 之功效，結果發現氮素與碳素源均會影響兩拮抗菌的抑病效率。在 24 種氮素源中，大多數的氨基酸皆可提昇 *P. polymyxa* Y-NYDA-21 菌株的抑病效率，惟僅有少數氨基酸可促進 *B. cereus* D-NYDA-602 菌株的抑病效果。此外，選擇十二種氨基酸培養兩菌株七天後，也發現大部份的氨基酸皆可提昇 *P. polymyxa* Y-NYDA-21 抑制百合灰黴病的病斑產生，惟只有 DL-ornithine 和 DL-tyrosine 可有效促進 *B. cereus* D-NYDA-602 降低灰黴病病斑數的能力。至於十一種碳素源中，則以 D(+)-glucose、α-lactose hydrate、D(+)-mannose 和 D(-)-ribose 等四種能顯著促進兩株拮抗菌的抑病效果。比較不等量之 DL-ornithine 與 D(+)-glucose 或 D(+)-mannose組合配方的防治病害效果，結果顯示 1%(w/v) DL-ornithine 分別與 0.5% (w/v) D(+)-glucose 或 1%(w/v) D(+)-mannose 之組合最能抑制百合灰黴病之發生。進一步，在溫室與田間利用 1%(w/v) DL-ornithine 與 1%(w/v) D(+)-mannose 培養 *P. polymyxa* Y-NYDA-21 七天之稀釋培養液、過濾液及菌體等防治百合灰黴病，結果顯示三者均具有減輕百合灰黴病發生的功效。

關鍵詞：碳素源、氮素源、拮抗細菌、百合灰黴病菌、生物防治

緒　言

百合灰黴病的病原菌 *Botrytis elliptica* (Berk.) Cooke，可引起百合莖、葉及花苞等部位出現紅褐色圓形至橢圓形病斑，發病嚴重時葉片病斑癒合，造成葉枯現象，並使觀賞及商品價值頓減 [4,5]。綜觀近二十年的文獻記載，防治百合灰黴病的主要方法係採用化學藥劑，惟效果不彰，且易出現農藥殘留與產生菌株抗藥性的問題 [11,23,24,30,33]。因此，許多學者嘗試利用拮抗菌進行作物灰黴病 (*Botrytis cinerea* Pers. ex Fr.) 的生物防治，例如 Blakeman 與 Fokkema [10] 以植物葉表微生物如真菌，酵母菌或細菌等噴施於作物上，可顯著降低作物灰黴病的發生。此外，也有利用 *Trichoderma* spp. 和 *Gliocladium* spp. 防治葡萄、草莓和蘋果灰黴病的報導 [7,14,15,21,32,35,36]。最近 Elad 氏等 [16] 成功地利用酵母菌 *Rhodotorula glutinis* 和 *Cryptococcus albidus* 抑制灰黴病菌感染豆類與番茄及降低其產孢作用。劉氏 [3] 報導五株木黴菌 *Trichoderma* spp. 和一株酵母菌 *Cryptococcus* sp. 噴於草莓果實上，可顯著降低灰黴病菌的為害。然而，拮抗微生物能否有效防治植物病害，常與其培養基組成配方與醱酵流程有關 [20]。倪氏 [1] 發現培養基中的碳素和氮素源會左右 *B. subtilis* PB-113 對 *Erwinia herbicola* 的抗生作用。鍾和黃氏 [6] 亦曾指出麩胺酸與蔗糖兩者可促進 *Bacillus* spp. BR-11 與 BS-25 拮抗 *Alternaria brassicicola*。本研究的主要目的在於探討氮和碳素營養源對拮抗細菌抑制百合灰黴病的影響，祈能明瞭營養源在生物防治體系中扮演的角色，藉以作為研發微生物製劑配方防治百合灰黴病的基礎。

材料與方法

菌株來源、產孢與保存

將百合灰黴病菌 *B. elliptica* B066 菌株 [25] 培養於馬鈴薯葡萄糖瓊脂 (potato dextrose agar, PDA, Difco Laboratory,

Detroit, MI, USA) 斜面上，置於 20℃ 黑暗的定溫箱中培養，待菌絲長滿後，切取小菌絲塊移入 10% V-8 培養基平板上 [V8 juice agar: V8 juice (Campbell Soup Co., Camden, NJ, USA) 100 ml, $CaCO_3$ (Sigma Chemical Co., St. Louis, MO, USA) 2g, agar 20g, distilled water 900 ml]，或移入高壓 (121℃、15 lb/in.^{2}) 滅菌過之百合花瓣上，並移置於 20℃定溫箱內，於距 7 cm 近紫外光燈 (黑燈管 Black Light Blue, F10T8BLB, Sankyo Denki, Japan) 下，每天照射 12 小時，7 至 10 天後，*B. elliptica* B066 即可大量產孢。隨後按 Doss 氏等 [13] 保存菌種的方法，將 B066 保存在 5℃的燕麥粉砂 (3% oatmeal in fine silica sand) 中備用。接種時，以無菌水將 V-8 培養基平板上或百合花瓣上產孢之灰黴病菌孢子洗下，經二層紗布過濾，再以離心機 (Sigma 3K12, Laboratory centrifuges, Martin Christ, West Germany) 3000 X *g* 離心 10 分鐘，去除上層液，加入無菌水再離心，連續三次後，加水調整至所需之濃度。

拮抗細菌之來源與鑑定

將百合灰黴病的拮抗細菌 D-NYDA-602 和 Y-NYDA-21 菌株 [25]，培養於 Nutrient agar (NB, Difco Laboratory, Detroit, MI. USA) 平板上，在 30℃ 培養 24 小時後，以 3% KOH 溶液進行革蘭氏染色測定，結果 D-NYDA-602 及 Y-NYDA-21 菌株皆屬於革蘭氏陽性桿菌。將兩菌株劃線培養於 BUG^{TM}＋M＋T (0.25% maltose 及 0.9% thioglycolate) 培養基平板上，在 30℃ 上培養 20 小時，經多次更新培養後，以無菌棉棒沾取菌體，使其懸浮於 25 ml 的 GN/GP-IF (Biolog's GN/GP inoculating fluid) 溶液中，使細菌濃度調整為 28% T±3%，然後接種到 Biolog GP2 Microplate^{TM}，每穴加入 150 µl 細菌懸浮液，放置在 30℃ 培養 4-16 小時後，以 Biolog reader 檢視測試菌株對 95 種碳素源之利用情形，並以 Biolog's MicroLog^{TM} computer Sofeware (Biolog MicroLog^{TM} system, Release 4.0) 測試和判斷菌株之菌屬與種名相似度。

氮及碳素源影響拮抗細菌防治百合灰黴病之效果

將 Czapek-Dox solution 組成配方 (5g $NaNO_3$、5g Sucrose、1g K_2HPO_4、0.5g $MgSO_4 \cdot 7H_2O$、0.5g KCl、0.01g $FeSO_4$、1 mL 1% $ZnSO_4$、1 mL 0.5% $CuSO_4$、10 mL Hoagland's solution、1 L Distilled water) 中的氮素源 $NaNO_3$ 以各種氨基酸取代，其中氨基酸分別為 DL 型的 alanine、arginine HCl、asparagine、aspartic acid、citrulline、cystine、β-3,4-dihydroxyphenylalanine、glutmic acid、glycine、histidine HCl、isoleucine、leucine、lysine HCl、methionine、norvaline、norleucine、ornithine、phenylalanine、proline、serine、threonine、tryptophan、tyrosine、valine) (Sigma) 等 21 種，此外，並以不添加氮素源為對照組。經滅菌後，在 49 ml 的修正培養基中，分別接種 1 ml D-NYDA-602 及 Y-NYDA-21 細菌懸浮液 (10^8 cfu/ml)，分別培養四和七天後，利用葉片圓盤生物分析法[25]，測定拮抗菌抑制百合灰黴病的效果。生物分析方法是：將百合葉片圓盤 (15 mm 直徑)之葉背浸泡於拮抗菌培養液 (每毫升約含 3×10^8 cfu 細菌) 的 10 倍、100 倍及 1000 倍稀釋液中，3 分鐘後取出葉片圓盤晾乾，隨後接種每毫升含 10^5 灰黴菌孢子懸浮液，保持 100% 相對濕度，置於 20℃ 定溫箱中，一天後記錄水浸狀病斑數目，三天後觀察並記錄葉片圓盤上罹病斑百分率。進一步再選取其中較優良的 12 種氮素源，分別培養拮抗菌後，亦以葉片圓盤法評估各別的抑病效果。隨後選 0.5% (w/v) DL-ornithine 充作 Czapek-Dox solution 中的氮源，分別以 0.5% (w/v) D(-)-arabinose、β-D(-)-fructose、D(+)-galactose、D(+)-glucose、β-D(+)-gluose、α-lactose hydrate、maltose hydrate、D(+)-mannose、D(-)-ribose、sucrose 及 D(+)-xylose (Sigma) 等 11 種碳源替代培養液配方中之蔗糖，製成不同碳源培養液後，接種兩拮抗菌培養第四與第七天時，按上述方法評估碳素源對拮抗菌防治灰黴病之影響。其中並以不添加任何碳素源為對照組。最後將試驗獲得結果之百合葉片圓盤罹患灰黴病百分率，採下列公式轉換成拮抗菌的抑病比值。

$$\text{抑病比值 \%(Inhibition ratio)} = \frac{S-N}{S}\times 100$$

S 為去氮 (-N) 或去碳 (-C) 素源對照組的罹病率，N為各種氮或碳素源處理組之罹病率。

氮及碳素源不同比例對百合灰黴病的影響

選取氮素源 DL-ornithine 與碳素源 D(+)-glucose 或 D(+)-mannose 以 0、0.125、0.25、0.5 和 1.0%(w/v) 之量相互混合，組成各種碳氮源不同含量比之營養液，分別處理百合葉片圓盤後，再行接種百合灰黴病菌，藉以評估不同組合營養源對灰黴病發生之影響。

兩種氮碳源新組合營養液對拮抗細菌防治百合灰黴病之影響

取 1%(w/v) DL-ornithine 和 0.5%(w/v) D(+)-glucose，1%(w/v) DL-ornithine 和 1%(w/v) D(+)-mannose 等二種組合營養液分別培養拮抗細菌 24-2-2、49-2-4、B-NA-12、B-NYDA-10、B-PDAS-21、D-NYDA-602 和 Y-NYDA-21 等菌株 (註：1996-1997 年自百合葉片分離獲得，對灰黴病菌具拮抗作用，保存於行政院農委會農試所植病系花卉病害研究室)七天後，將各拮抗細菌培養液 (每毫升約含 3×10^8 cfu 細菌) 稀釋成 10、100 和 1000 倍懸浮液，分別處理百合葉片圓盤後，測試兩種組合營養液影響拮抗細菌防治

百合灰黴病之效果。每一處理有八重複，以不含拮抗細菌之含氮和碳營養液作為對照組。接種後一天記錄百合葉片圓盤出現病斑的數目。

溫室試驗

利用 1%(w/v) DL-Ornithine 與 1%(w/v) D(+)-Mannose 作為 Czapek-Dox solution 之氮／碳素源 (簡稱 OM solution)，培養 *P. polymyxa* Y-NYDA-21 七天後的培養液、過濾液及菌體等分別稀釋10倍，然後在溫室中 (24±4 ℃)，噴佈於露蕾期葵百合 (cv. Star Gazer) 植株 (株齡一個半月至二個月)葉片上，其中並以未培養拮抗菌之營養液及自來水作為對照組，每處理五重複。待葉片風乾後，再行接種 5×10^4 conidia/ml 之灰黴病菌孢子懸浮液於植株葉背上，每株約接種 10 ml，套上透明塑膠袋保濕，置於 20 ℃、相對濕度 90%、光照 16 小時、照度 3500 lux (Digital illumination meter, DX-200, Jing Chen Instruments Co., Taichung) 之生長箱中，二天後去除塑膠袋，於第七天記錄第 5 至 8 位葉之病斑數。本試驗重複進行二次。

田間試驗

西元 1998 年 2 月至 3 月間，在台中縣新社鄉行政院農委會種苗改良繁殖場之百合灰黴病罹病田進行田間防治試驗。採完全逢機區集設計，將種植 1.5 個月的東方型百合 Acapulco 品種之栽培田各劃分成四個區集，每區集計有五小區，大小為 5.0m×0.6m。五小區百合分別處理以 OM solution 培養 *P. polymyxa* Y-NYDA-21 七天之稀釋 10 倍培養液 (每毫升約含 3×10^7 cfu 細菌)、過濾液及菌體等；同時也以未培養拮抗菌之營養液及自來水作為對照組，每處理四重複。每星期噴施一次，連續三次，於每次噴施前及最後一次噴施後一星期記錄百合之罹病度 (disease severity)。罹病度之計算係將葉片罹病面積分成 4 級：1＝1－10% 葉片面積罹病；2＝11－25% 面積罹病；3＝26－50% 面積罹病，4＝51% 以上面積罹病，然後依下列公式計算罹病度。

$$\text{罹病度 (\%)} = \frac{\Sigma\ (\text{罹病級數 X 該級數之葉數})}{\text{調查總葉數}\times 4}\times 100$$

結　果

拮抗細菌之鑑定

拮抗細菌 D-NYDA-602 及 Y-NYDA-21 兩菌株經由 Biolog GP2 Microplate™ 測定 4 小時與 16 小時後，以 Biolog's MicroLog™ Computer Sofeware 進行分析，獲得的數據與資料庫中的細菌資料比對，鑑定 D-NYDA-602 菌株為 *Bacillus cereus*，Y-NYDA-21 菌株為 *Paenibacillus polymyxa*。

氮及碳素源影響拮抗細菌防治百合灰黴病之效果

氨基酸 (氮素源) 會影響 *B. cereus* D-NYDA-602 和 *P. polymyxa* Y-NYDA-21 兩拮抗菌抑制灰黴病的效果。各氮素營養源培養兩拮抗菌，在第四天時，僅 DL-lysine HCl 和 DL-norleucine 可提昇 *B. cereus* D-NYDA-602 的防病效率，惟多數氨基酸均可增加 P. polymyxa Y-NYDA-21 菌株的抑病率，其中以 DL-aspartic acid、DL-cystine、DL-histidine HCl、DL-norvaline、DL-norleucine 和 DL-ornithine 的效果最佳；而培養至第七天時，大多數氨基酸均可提昇兩株拮抗菌的防病效率，其中僅 DL-leucine 和 DL-valine 無法有效促進 *B. cereus* D-NYDA-602 的防病率；而 DL-arginine HCl、DL-cystine、DL-phenylalanine、DL-tryptophan 和DL-tyrosine 等也不具有促進 *P. polymyxa* Y-NYDA-21 的防病功效 (表一)。

由表一之結果，選取 DL-alanine、DL-asparagine、DL-aspartic acid、DL-histidine HCl、DL-lysine HCl、DL-methionine、DL-norvaline、DL-norleucine、DL-ornithine、DL-proline、DL-serine 和 DL-tyrosine 等十二種氨基酸，進一步測試它們對兩株拮抗菌抑制灰黴病的影響。結果只有 DL-ornithine 和 DL-tyrosine 可促使 *B. cereus* D-NYDA-602 顯著降低百合灰黴病的病斑數；然而除 DL-alanine 外，所有氨基酸卻皆可有效增加 *P. polymyxa* Y-NYDA-21 減少灰黴病病斑數的能力 (表二)。綜合上述結果發現兩菌株在不同氮素源營養液中培養後，以 *P. polymyxa* Y-NYDA-21 抑制百合灰黴病的效果較佳 (表一、二)。

由於 DL-ornithine 能有效增強兩拮抗菌株的抑病效果 (表二)，故以其替代 Czapek-Dox solution 中的 $NaNO_3$，隨後添加各種碳素源，探討不同碳源對兩株拮抗菌防病的影響。結果培養四天時，所有的碳素源均無法有效促進兩株拮抗菌的防病效率。惟培養七天時， D(-)-arabinose、D(+)-galactose、D(+)-glucose、β-D(+)-glucose、α-lactose hydrate、maltose hydrate、D(+)-mannose 和 D(-)-ribose等八種碳素源均可增進 *B. cereus* D-NYDA-602 的防病功效；至於 D(+)-glucose、α-lactose hydrate、D(+)-mannose、D(-)-ribose 及 D(+)-xylose 等五種碳素源亦可增進 *P. polymyxa* Y-NYDA-21 的防病率。綜合兩組結果，發現 D(+)-glucose、α-lactose hydrate、D(+)-mannose 及 D(-)-ribose 等四種均可有效促進兩拮抗菌的抑病功效 (表三)。

氮及碳素源不同比例對百合灰黴病之影響

取 DL-ornithine 分別與 D(+)-glucose 或 D(+)-mannose 作氮／碳素源之不同量組合處理於百合葉片圓盤，測試它

表一、利用葉片圓盤法評估氮素源影響拮抗菌抑制百合灰黴病的效果

Table 1. Use leaf disk assay to evaluate the effect of nitrogen sources on the suppression of *Botrytis* leaf blight of lily by antagonistic microorganisms at 20℃.

Nitrogen source[1]	Inhibition ratio (%) [2]			
	4 days		7 days	
	D-602[1]	Y-21	D-602	Y-21
DL-Alanine	23 j-m[3]	19 c-g	48 d-l	35 b-f
DL-Arginine HCl	-64 bc	7 c-f	46 c-k	24 abc
DL-Asparagine	-39 cde	40 ghi	56 f-l	71 h
DL-Aspartic acid	30 klm	58 hij	66 jkl	59 fgh
DL-Citrulline	0 h-k	9 c-f	62 h-l	29 bcd
DL-Cystine	-7 e-j	72 j	34 b-g	16 ab
DL-β-3,4-Dihy droxy phenylalanine	-55 bcd	-2 cd	28 b-e	37 b-f
DL-Glutamic acid	-32 d-g	-7 bc	64 i-l	55 fgh
Glycine	-5 f-j	9 c-f	42 c-j	39 b-f
DL-Histidine HCl	-5 f-j	60 ij	48 c-k	55 e-h
DL-Isoleucine	-11 e-j	23 d-g	36 b-h	49 d-h
DL-Leucine	-16 e-i	40 ghi	12 ab	33 b-e
DL-Lysine HCl	34 lm	40 ghi	52 d-l	49 d-h
DL-Methionine	16 i-m	33 fgh	60 g-l	45 c-g
DL-Norvaline	-5 g-j	60 ij	76 l	45 c-g
DL-Norleucine	41 m	74 j	70 kl	63 gh
DL-Ornithine	20 j-m	74 j	34 b-g	41 c-g
DL-Phenylalanine	2 h-l	42 ghi	34 b-g	6 a
DL-Proline	-27 d-h	16 c-g	48 d-l	59 fgh
DL-Serine	-36 c-f	30 fg	54 e-l	59 fgh
DL-Threonine	9 i-l	-30 ab	28 b-e	31 b-e
DL-Tryptophan	-102 a	30 fg	38 c-e	16 ab
DL-Tyrosine	0 g-k	21 d-g	26 bcd	4 a
DL-Valine	-2 g-j	-37 a	22 abc	35 b-f
－N source (Control)	0 h-k	0 cde	0 a	0 a

1. Nitrogen source in the modified Czapek-Dox solution for culturing two antagonists, *Bacillus cereus*, D-NYDA-602 (D-602) and *Paenibacillus polymyxa* Y-NYDA-21 (Y-21).
2. Inhibition ratio (%)=(S-N or S)×100%/S, S and N represented the values of lesion areas in control and treated leaf disks, respectively.
3. Data followed by the same letter in each column do not differ significantly (p=0.05) according to Duncan's multiple range test.

們對灰黴病發生之影響，結果顯示葉片圓盤上罹病斑數會隨著氮和碳素源含量的增加而降低，其中以 1%(w/v) DL-ornithine 分別與 0.5%(w/v) D(+)-glucose 和 1%(w/v) D(+)-mannose 之組合最能有效抑制灰黴病之發生 (圖一、二)。

兩種氮碳源新組合營養液影響拮抗細菌防治百合灰黴病之效果

以含 1%(w/v) ornithine 與 0.5%(w/v) glucose (OG) 或含 1%(w/v) ornithine 與 1%(w/v) mannose (OM) 的營養液培養七種拮抗細菌七天後，分別以其 10、100 和 1000 倍稀

表二、氮素源影響拮抗菌降低百合灰黴病病斑數目的效果

Table 2. Effect of nitrogen sources on the producing lesion numbers of Botrytis leaf blight on lily by antagonistic microorganisms at 20℃

Nitrogen source [1]	Lesion numbers per leaf disk [2]	
	D-602 [1]	Y-21
DL-Alanine	22.0 a[3]	17.3 ab
DL-Asparagine	18.3 ab	8.1 efg
DL-Aspartic acid	17.4 ab	11.9 c-f
DL-Histidine HCl	14.9 bc	14.6 bc
DL-Lysine HCl	11.1 cd	6.4 g
DL-Methionine	17.1 ab	8.6 efg
DL-Norvaline	11.3 cd	9.0 efg
DL-Norleucine	11.1 cd	12.0 c-f
DL-Ornithine	5.0 e	7.6 fg
DL-Proline	11.4 cd	14.1 bcd
DL-Serine	11.5 cd	12.9 b-e
DL-Tyrosine	9.4 de	9.5 d-g
－N source (Control)	16.3 bc	20.8 a

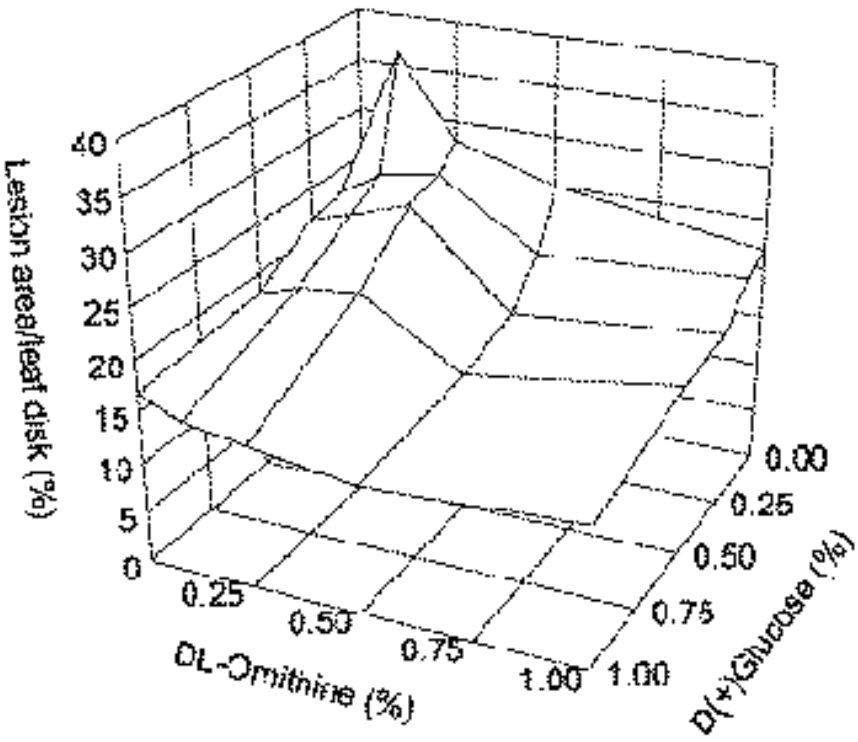
1. Nitrogen source in the modified Czapek-Dox solution for culturing two antagonists, *Bacillus cereus*, D-NYDA-602 (D-602) and *Paenibacillus polymyxa* Y-NYDA-21 (Y-21) for 4 days.
2. Data were recorded one day after inoculation.
3. Data followed by the same letter in each column do not differ significantly (p=0.05) according to Duncan's multiple range test.

表三、利用葉片圓盤法評估碳素源影響拮抗菌抑制百合灰黴病的效果

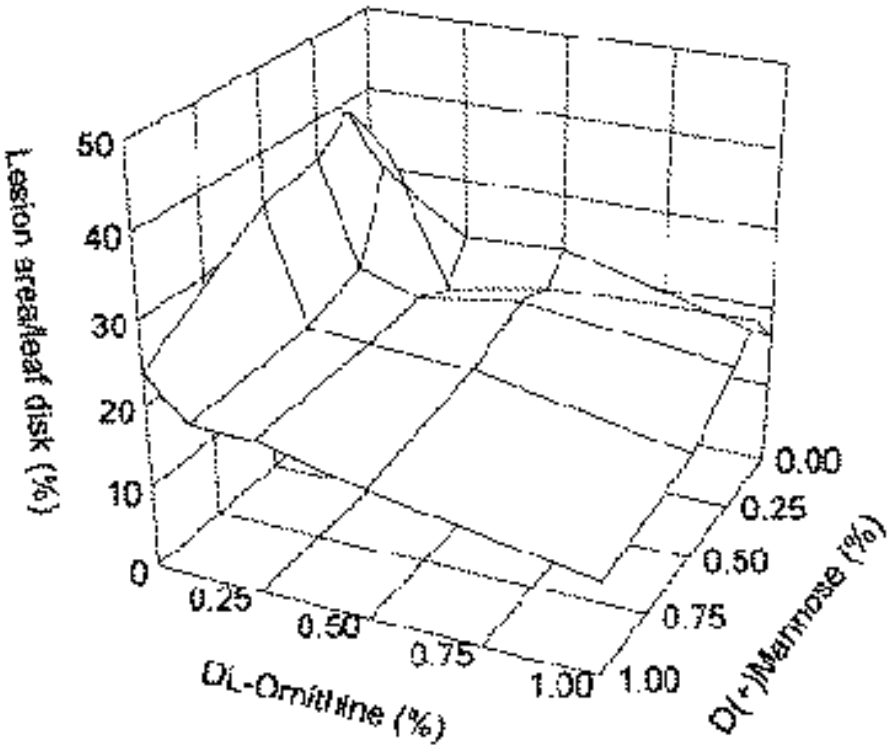
Table 3. Use leaf disk assay to evaluate the effect of carbon sources on the suppression of Botrytis leaf blight of lily by antagonistic microorganisms at 20℃

Carbon source [1]	Inhibition ratio (%) [2]			
	4 days		7 days	
	D-602[1]	Y-21	D-602	Y-21
D(-)-Arabinose	-13 cde[3]	43 cd	44 c	4 bc
β-D(-)-Fructose	-200 ab	-13 bc	11 b	18 bcd
D(+)-Galactose	0 de	35 cd	45 c	16 bcd
D(+)-Glucose	0 de	57 d	47 c	31 cd
β-D(+)-Glucose	-100 bcd	52 d	62 cd	24 bcd
α-Lactose hydrate	0 de	48 cd	42 c	31 cd
Maltose hydrate	13 e	57 d	51 c	7 bc
D(+)-Mannose	13 e	57 d	55 c	47 d
D(-)-Ribose	-113 bc	-83 a	75 d	42 d
Sucrose	-250 a	-48 ab	-24 a	-31 a
D(+)-Xylose	-38 cde	4 bcd	-29 a	44 d
－C source (Control)	0 de	0 bcd	0 b	0 b

1. Carbon source in the modified Czapek-Dox solution for culturing two antagonists, *Bacillus cereus*, D-NYDA-602 (D-602) and *Paenibacillus polymyxa* Y-NYDA-21 (Y-21) for 4 days.
2. Inhibition ratio (%)=(S-N or S)×100%/S, S and N represented the values of lesion areas in control and treated leaf disks, respectively.
3. Data followed by the same letter in each column do not differ significantly (p=0.05) according to Duncan's multiple range test.



圖一、利用百合葉片圓盤法評估 Czapek-Dox 營養液中含有不同濃度的鳥胺酸和葡萄糖對百合灰黴病發生的影響。
Fig. 1. Use leaf disk method to evaluate the effect of concentrations of ornithine and glucose in the modified Czapek-Dox solution on severity of lily leaf blight caused by *Botrytis elliptica*.



圖二、利用百合葉片圓盤法評估 Czapek-Dox 營養液中含有不同濃度的鳥胺酸和甘露糖對百合灰黴病發生的影響。
Fig. 2. Use leaf disk method to evaluate the effect of concentrations of ornithine and mannose in the modified Czapek-Dox solution on severity of lily leaf blight caused by *Botrytis elliptica*.

釋液防治百合灰黴病，結果顯示除 B-NYDA-10 和 B-PDAS-21 兩菌株外，OG 營養液培養各拮抗菌的 10 倍稀釋液，均可有效抑制病害的發生；稀釋 100 倍者，則以 24-2-2、D-NYDA-602 和 Y-NYDA-21 最能抑制百合圓盤上的病斑數目；至於稀釋1000倍時，各菌株均無法有效降低灰黴病的發生 (表四)。在 OM 營養液培養各拮抗菌的 10 倍稀釋液，除 B-PDAS-21 外，其餘菌株均可有效降低病斑的數目，其中以 Y-NYDA-21 菌株的效果最佳；此外 OM 培養拮抗菌之 100 或 1000 倍稀釋液，各拮抗菌株亦均能有效降低百合葉片的病斑數目 (表四)。

溫室與田間試驗成果

利用 1%(w/v) DL-ornithine 與 1%(w/v) D(+)-mannose (OM solution) 培養 *P. polymyxa* Y-NYDA-21 七天之培養液、過濾液及菌體等 10 倍稀釋液，在溫室中測試各別防治灰黴病之效果，結果顯示拮抗細菌之培養液、過濾液及菌體等三者稀釋液均能有效減輕灰黴病之發生。其中以拮抗細菌之培養液最能降低百合葉片的罹病斑率，惟在統計分析上，與過濾液和菌體稀釋液防治效果間無顯著差異 (表五)。

於田間自然發病田試驗 OM 營養液培養 *P. polymyxa* Y-NYDA-21 防治百合灰黴病的效果，發現每隔一星期噴施一次 OM 營養液培養之細菌液、過濾液和菌體稀釋液，連續噴施三次後，結果顯示前三次調查之百合病害罹病度與對照組間無顯著差異性，直至處理第三次後一星期，即第四次調查結果，才顯示拮抗細菌之培養液、過濾液與菌體稀釋液等三者具有較對照組顯著 (p=0.05) 降低病害發生的功效 (表五)。

討 論

植物葉片可分泌出一些有機與無機物質，如氨基酸、單多醣類、有機酸、醣醇及一些巨量與微量元素 [37]。葉表泌出之營養可直接影響拮抗微生物與病原菌間的相互作用 [31]。高量的營養可促進葉表微生物之活力及延續其在葉片上存活的時間 [9]。多位學者指出添加營養促進葉表酵母菌之活力可有效防治植物病害 [8,18,31]。又噴佈醣蜜於植物體，可促進 *Sporobolomyces* spp.、*Cryptococcus* spp.、*Aureobasidium pullulans* 和 *Cladosporium cladosporioides* 等菌類族群的繁殖 [12,19]。2%(w/v) 氯化鈣水溶液可促進酵母菌 *Kloeckera apiculata* 防治蘋果灰黴病及青黴病之功效 [29]。噴佈 CH100 植物健素 300 倍液於韭菜上，可增加葉表酵母菌及其他真菌總量達 3-10 倍左右 [2,26]。鍾和黃兩氏 [6] 由十六種氮素與十種碳素源選出麩胺酸 (glutamic acid) 與蔗糖 (sucrose) 二者可增進 *Bacillus* spp. BR-11 與 BS-25 兩株菌拮抗十字花科蔬菜黑斑病菌 (*Alternaria brassicicola*) 的能力，以上結果顯示營養源確可左右拮抗菌在葉表上的抑病功效。

本研究測試氮素與碳素源影響拮抗細菌抑制百合灰黴病的效果，發現拮抗細菌生長於不同的氮與碳素源營養液後，分別對灰黴病的病勢進展具有不等的影響效應。其中氮素源如 DL-alanine、DL-aspartic acid、DL-lysine HCl、DL-methionine、DL-norleucine、DL-ornithine、DL-phenylalanine 和 DL-threonine 等八種氨基酸皆會影響兩拮抗菌株抑制灰黴病的功效，尤其是 DL-ornithine能顯著增強兩拮抗菌株之抑病效果。在受測的十一種碳素源中，發現 D(+)-glucose、α-lactose hydrate、D(+)-mannose 及 D(-)-ribose等四種可以提高拮抗菌抑制灰黴病的效果。

眾所周知，*Bacillus* spp. 會產生多種抗生物質，其中

表四、兩種氮和碳素源組合營養液影響拮抗菌抑制百合灰黴病的效果比較

Table 4. Effects of two combinations of nitrogen and carbon sources on reducing the lesion numbers of Botrytis leaf blight on leaf discs by various antagonists

Antagonist no.	Lesion numbers per leaf disk[1]					
	OG[2]			OM[2]		
	10×	100×	1000×	10×	100×	1000×
24-2-2	5 de[4]	18 de	23 ab	25 bc	18 bc	21 bc
49-2-4	17 bc	19 cde	24 ab	16 cde	18 bc	16 c
B-NA-12	3 e	31 ab	29 a	19 cde	14 c	20 bc
B-NYDA-10	24 ab	30 abc	17 b	21 cd	24 b	19 bc
B-PDAS-21	29 a	38 a	25 ab	32 ab	27 b	30 b
D-NYDA-602	14 cd	15 e	16 b	20 cd	19 bc	21 bc
Y-NYDA-21	17 bc	15 e	16 b	9 e	14 c	16 c
Control (None)[3]	29 a	29 abc	27 ab	39 a	39 a	40 a

1. Lesion number was evaluated with leaf disk method [25].
2. OG: 1%(w/v) ornithine + 0.5%(w/v) glucose; OM: 1%(w/v) ornithine + 1%(w/v) mannose; 10X, 100X, and 1000X indicating dilution times of the nutrient solution.
3. Each nutrient solution without antagonist was used as a control.
4. Data followed by the same letter in each column do not differ significantly (p=0.05) according to Duncan's multiple range test.

表五、在溫室及田間利用拮抗菌 *Paenibacillus polymyxa* Y-NYDA-21防治百合灰黴病的效果

Table 5. Effect of *Paenibacillus polymyxa* Y-NYDA-21 on control of lily leaf blight caused by *Botrytis elliptica* in the greenhouse and field trials

Treatment [1]	Disease severity [2] (%)				
	Greenhouse trial	Field trial			
		Feb. 26	Mar. 5	Mar. 12	Mar. 19
B	15.8 b[3]	1.8 ab	9.8 ab	13.4 a	24.6 b
S	14.6 b	2.8 a	11.2 ab	15.8 a	28.0 b
BS	9.5 b	0.8 b	7.0 b	12.6 a	24.8 b
OM	24.0 a	1.4 ab	16.8 a	18.8 a	37.6 ab
Water(Check)	22.7 a	1.6 ab	14.4 ab	21.6 a	43.8 a

1. OM solution = Modified Czapek-Dox solution contained 1% (w/v) DL-ornithine and 1% (w/v) D(+)mannose, BS = Antagonist Y-21 cultured in OM solution for 4 days at 24℃, BS was separated into B (mass of antagonists) and S (cultural solution) by centrifugation at 3000 × *g* for 10 min.
2. Disease severity in the greenhouse and field trials were assessed by counting lesion numbers per leaf and by leaf infected areas, respectively. Scale (0-4) of infected leaf. 0= no lesion was observed, 1= 1-10% leaf areas was infected, 2= 11-25% leaf areas was infected, 3= 26-50% leaf area was infected, and 4= over 50% leaf area was infected.
3. Data followed by the same letter in each column do not differ significantly (p=0.05) according to Duncan's multiple range test.

以胜肽類為主 (28)，而有些氨基酸可參與該菌抗生物質的生合成 (22,27)。倪氏 (1) 在培養基中添加 alanine、arginine、aspargine、aspartic acid、citrulline、glutamic acid、glutamine 和 proline 等可促進 *B. subtilis* PB-113 菌株產生抗生物質。本研究也發現多種氨基酸均可有效促進拮抗細菌抑制灰黴病發生的功效。

拮抗菌培養時間可影響其防治病害的效果。鍾和黃兩氏 (6) 發現在蔗糖麩胺酸培養液中，*Bacillus* spp. BR-11 和 BS-25 菌株之代謝物質的拮抗效果隨著培養時間的增加而增加。Stroke 和 Woodward (34) 與 Feeney 氏等 (17) 亦指出培養的時間會影響拮抗菌產生抗生物質的量與種類，進而影響其防病的效力。本試驗發現拮抗菌在不同氮素源培養液中培養七天時的抑病效果最好。碳素源方面，*B. cereus* D-NYDA-602 在各碳素營養液中培養一天後的抑病能力較 *P. polymyxa* Y-NYDA-21 為佳 (數據未列於表中)，然而培養四天後，反而以 *P. polymyxa* Y-NYDA-21 抑病的效果較佳。此說明不同拮抗菌株在碳氮素源營養液中的培養時間可影響其防病功效。採用 OM 營養液 (含 1% ornithine 與 1% mannose) 培養 *P. polymyxa* Y-NYDA-21 菌株七天時，其 10 倍稀釋液 (細菌菌量約為 3×10^7 cfu/ml)，可有效降低百合葉片圓盤出現灰黴病的病斑數目。溫室試驗發現拮抗細菌之培養液、過濾液與菌體稀釋液均能有效減輕灰黴病之發生。田間試驗結果亦佐證連續噴施三次拮抗細菌 *P. polymyxa* Y-NYDA-21 的培養液、過濾液與菌體稀釋液，可有效降低灰黴病的罹病度。綜合上述成果，證明氮和碳素源確可干擾拮抗細菌防治百合灰黴病的效果，因此，在調製微生物製劑配方時，慎選適於拮抗菌增殖與兼具促進抗生作用之碳氮素源，確是一項不可忽視的工作。

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ABSTRACT

Hsieh, T. F. [1], Huang, J. W. [2,4], Chang, C. C. [3], and Peng, Y. H. [2] 2001. Effect of nitrogen and carbon sources on biocontrol of lily leaf blight by *Bacillus cereus* and *Paenibacillus polymyxa*. Plant Pathol. Bull. 10:79-87. ([1.] Department of Plant Pathology, Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan, Wu-feng 413, Taichung, Taiwan; [2.] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; [3.] Department of Crop Environment, Taoyuan District Agricultural Improvement Station, Council of Agriculture, Executive Yuan, Hsin-wu 327, Taoyuan, Taiwan; [4.] Corresponding author, E-mail: jwhuang@dragon.nchu.edu.tw; Fax: 886-2-22851676)

The effect of various carbon and nitrogen sources on antagonistic efficiency of two antagonists, *Bacillus cereus* D-NYDA-602 and *Paenibacillus polymyxa* Y-NYDA-21, for control of lily leaf blight caused by *Botrytis elliptica* was evaluated using the leaf disk assay, greenhouse tests, and field trials. Twenty-four nitrogen or 11 carbon sources were respectively utilized to replace $NaNO_3$ or sucrose of a modified Czapek-Dox solution formula and to culture two biocontrol agents for seven days. The results indicated that the inhibition effect of the two biocontrol agents on lily leaf blight could be markedly influenced by several nitrogen and carbon sources in their growth substrates. Among the 24 amino acids tests, most of them were able to increase ability of *P. polymyxa* Y-NYDA-21 to decrease the disease severity, however only a few could enhance suppressive ability of *B. cereus* D-NYDA-602 in leaf disk assays. In advance tests, 12 amino acids were selected to evaluate for their effectiveness in culturing two biocontrol agents. It was found that both DL-ornithine and DL-tyrosine were significantly effective in enhancing the antagonistic ability of *P. polymyxa* Y-NYDA-21 and *B. cereus* D-NYDA-602 against *B. elliptica* to induce lesion numbers in lily leaf disks. Among 11 carbon sources tested, D(+)-glucose, α-lactose hydrate, D(+)-mannose, and D(-)-ribose were proved to make the biocontrol agents showing stronger antagonistic effect. Therefore, DL-ornithine, D(+)-glucose, and D(+)-mannose were elected as N- and C-source components of the modified Czapek-Dox solution for cultivation of two biocontrol agents. The nutrient solution consisted of 1%(w/v) ornithine and 0.5%(w/v) glucose (OG solution) or 1%(w/v) mannose (OM solution) was effective in reducing lesion numbers of the disease. To conduct the disease control trials in the greenhouse and field, *P. polymyxa* Y-NYDA-21 was cultured in the OM solution for seven days. The culture solution was separated into filtrate and biomass for study. The results indicated that three treatments including cultured solution, filtrate, and biomass could reduce the disease severity of lily leaf blight.

Key words: Antagonistic bacteria, biocontrol, *Botrytis elliptica*, carbon source, nitrogen source, and nutrient effect.