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Burkholderia gladioli 引起之杏鮑菇細菌性腐敗病

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摘　要

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杏鮑菇爲臺灣新興的食用菇類，近年來由於大規模栽培，使得許多病害相繼發生，造成業者之損失。本研究於 2008 年自臺中市大里區的杏鮑菇栽培場獲得一疑似細菌危害之菇體樣本，在其蕈傘及蕈柄處可見組織崩解、凹陷之病徵，患部經擠壓後有菌泥溢出。自罹病組織進行病原菌分離純化後獲得三菌株，經生理生化測試結果顯示該菌爲革蘭氏陰性菌，在 nutrient agar 與 King's B 培養基上形成白色菌落並產生不具螢光特性的黃色色素，此外，此細菌可於 41℃ 下生長，具有水解明膠、脂質、幾丁質、酪蛋白等分子的能力與氧化酶、觸酶等酵素活性，再以脂肪酸圖譜分析 (FAME)、Biolog GN2 Microplate™ 及 16S-23S 內轉錄區域 (internal transcribed sequence, ITS) 之序列分析進行鑑定，確認該菌爲 *Burkholderia gladioli*。並以杏鮑菇進行接種試驗，確認其病原性。根據病原菌特性及其造成的病徵，將 *B. gladioli* 在杏鮑菇上造成的病害定名爲杏鮑菇細菌性腐敗病，此爲 *B. gladioli* 在臺灣造成杏鮑菇細菌性腐敗病之首次報導。

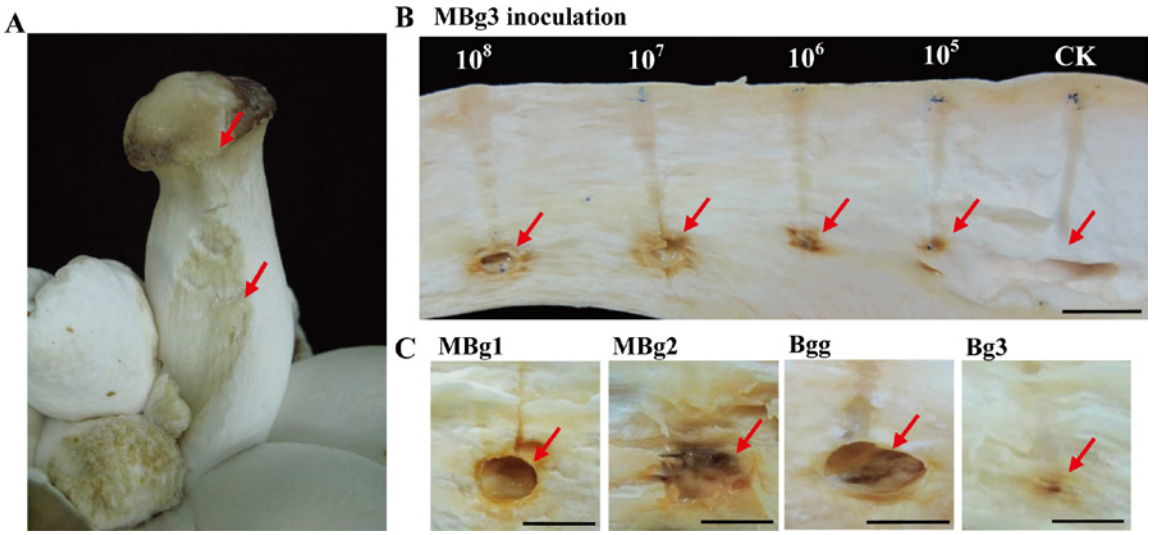
關鍵詞：杏鮑菇細菌性腐敗病、脂肪酸圖譜分析、16S-23S 內轉錄區域之序列分析

杏鮑菇 (*Pleurotus eryngii* [DC. ex Fr.] Quèl) 爲眞菌門 (Eumycota)、擔子菌亞門 (Basidiomycotina)、眞擔子菌綱 (Eubasidiomycetes)、帽菌亞綱 (Hymenomycetidae)、傘菌目 (Agaricales)、側耳菌科 (Pleurotaceae)、蠔菇屬 (*Pleurotus*) 的可食性菇類，廣泛分佈於南歐 (如法國、捷克斯拉夫平原、匈牙利、中亞地區)、北非及北美等地區[14, 15]。杏鮑菇因具有杏仁味且口感似鮑魚而得名，其蕈傘及蕈柄的質地、風味及烹調特性均較其他鮑魚菇佳，深受消費者的喜愛[14, 15]。近年來，臺灣杏鮑菇的栽培量僅次於香菇及金針菇，年產值近 10 億元[20]，已成爲臺灣重要的食用菇作物之一，但大規模商業化的栽培管理方式容易導致病害的發生，進而影響杏鮑菇的產量及品質、造成重大經濟損失。根據前人研究，已知可危害杏鮑菇的病原微生物包括杏鮑菇白黴病菌 (*Cladobotryum varium*)[1]、杏鮑菇褐腐病菌 (*Gliocladium roseum*)[3] 等眞菌病原與杏鮑菇褐色條斑病菌 (*Pseudomonas tolaasii*)[4]、杏鮑菇軟腐病菌 (*Pantoea* sp.)[8] 等細菌病原。

本研究於 2008 年自臺中市大里區 (舊行政區名爲臺中縣大里市) 的杏鮑菇栽培場獲得一疑似細菌性病原危害之菇體樣本，在其蕈傘及蕈柄處可見組織崩解、凹陷之病徵，擠壓病徵處有菌泥溢出 (圖一、A)。切取病組織於光學顯微鏡下觀察，發現有明顯細菌菌流的現象，以移殖環沾取菌液並利用劃線平板方式於 nutrient agar (NA, Difco Laboratories)、King's B medium (KB, 每公升含 20 g Bacto™ Proteose Peptone No. 3, 0.4 g $MgSO_4 \cdot 7H_2O$, 1.5 g K_2HPO_4, 10 ml glycerol) 及 potato dextrose agar (PDA, Difco Laboratories) 培養基進行病原

細菌之分離與培養。所分離之菌株在 NA 培養基上形成白色、表面具皺褶之菌落，此白色菌落在 KB 培養基上不會產生螢光物質，在 PDA 培養基上則呈現白色黏稠狀之菌落，此外生長於 KB 及 PDA 培養基上之菌落可分泌黃色色素。根據菌落型態與細菌培養特性，初步判斷本研究分離自杏鮑菇之病原細菌非螢光菌 *P. tolaasii*[4] 或黃色細菌 *Pantoea* sp.[8]，與前述報導中可感染杏鮑菇之病原細菌不同。

爲鑑定杏鮑菇致病菌的分類地位，首先以脂肪酸圖譜分析系統 (MIDI Sherlock® Microbial Identification System, MIS) 與氣相層析質譜儀 GC-MS (HP 6890N, USA) 分析杏鮑菇分離菌株 MBg1、MBg2 及 MBg3 的脂肪酸組成，結果顯示菌株 MBg1、MBg2 及 MBg3 的脂肪酸組成包含 C10:0 3OH、C14:0 3OH、C16:0、C16:0 2OH、C16:0 3OH、C16:1 2OH、C17:0 cyclo、C18:1 2OH 等碳氫鏈組合，與資料庫比對後，鑑定菌株 MBg1、MBg2 及 MBg3 爲 *Burkholderia gladioli*，其相似值分別爲 0.853、0.830 及 0.872。再以 Biolog GN2 Microplate™ (Biolog Inc. Hayward, CA, USA) 分析菌株 MBg1、MBg2 及 MBg3 對 95 種碳素源的利用情形，並以 Biolog Micrologg 3 ver. 4.20 系統進行分析與資料庫比對。結果顯示 MBg1、MBg2 及 MBg3 皆可利用其中 49 種碳素源：Tween 40、Tween 80、N-acetyl-d-glucosamine、adonitol、L-arabinose、D-arabitol、D-fructose、L-fucose、D-galactose、α-D-glucose、m-inositol、D-mannitol、D-mannose、D-psicose、D-sorbitol、D-trehalose、pyruvic acid methyl ester、succinic acid、mono-methyl-ester、acetic acid、cis-aconitic acid、citric acid、formic acid、D-gluconic acid、D-glucosaminic acid、α-hydroxybutyric acid、β-hydroxybutyric acid、α-keto butyric acid、D, L-lactic acid、quinic acid、D-saccharic acid、succinic acid、bromosuccinic acid、succinamic acid、D-alanine、L-alanine、L-alanyl-glycine、L-asparagine、L-aspartic acid、L-glutamic acid、L-histidine、hydroxy-L-proline、L-phenylalanine、L-proline、L-pyroglutamic acid、D-serine、L-serine、γ-amino butyric acid、D, L-α-glycerol phosphate、D-glucose-6-phosphate，確認菌株 MBg1、MBg2 及 MBg3 爲 *B. gladioli*，其相似值分別爲 0.84、0.76 及 0.58，與脂肪酸鑑定結果相符。



圖一、杏鮑菇細菌性腐敗病之病徵。
A、採集之子實體樣本，於蕈傘及蕈柄處呈現組織崩解、凹陷病徵；B、將杏鮑菇細菌性腐敗病菌 MBg3 調整爲 10^8 CFU/ml 之細菌懸浮液經 10 倍序列稀釋後穿刺接種於杏鮑菇蕈柄，並置 16 ℃ 生長箱 6 天後呈現組織凹陷及褐化等病徵，接種無菌水 (CK) 之對照組則無病徵產生；C、杏鮑菇細菌性腐敗病菌 MBg1、MBg2、唐菖蒲首腐病菌 (*Burkholderia gladioli* pv. *gladioli*, Bgg) 及鳳梨果腐病菌 (*B. gladioli* strain Bg3) 以 10^8 CFU/ml 穿刺接種於杏鮑菇蕈柄，並置於 16 ℃ 生長箱 6 天後的病徵。(Bar = 1 cm)

Fig. 1. Symptoms of bacterial decay disease on king oyster mushrooms. The red arrows indicate the decay tissues on cap (A) and stalk (A, B, and C) of the mushroom. Microtip-stab inoculation of *Burkholderia gladioli* strains MBg3 (B), MBg1, MBg2, *B. gladioli* pv. *gladioli* strain Bgg, and *B. gladioli* strain Bg3 (C) into the stalks of king oyster mushrooms are performed for pathogenicity test. (B) Bacterial inoculum of 10^8 CFU/ml is serially diluted, stabbed into the stalks of king oyster mushroom, and incubated at 16 ℃, 6 days for symptom observation. CK, mushroom stalks are stab-inoculated with sterile water as a control. (C) Bacterial inocula of 10^8 CFU/ml are used for inoculation. The pictures shown in B and C are taken at 6 days post inoculation. Bar =1 cm.

進一步利用前人設計之 16S-23S rDNA 內轉錄區域 (internal transcribed spacer region, ITS) 專一性引子對 primer 1 (5'-TTGTACACACCGC CCGTCA) 與 primer 2 (5'-GGTACCTTAGATGTTTCAGTTC) [9] 增幅菌株 MBg1、MBg2 及 MBg3 之 ITS 片段，得到一大小爲 868 bp 的片段，將此 DNA 片段送到中興大學生物科技發展中心進行定序，並利用美國國家生物科技資訊中心 (National Center for Biotechnology Information, NCBI. http://www.ncbi.nlm.nih.gov/) 進行基因庫比對與分析，結果顯示菌株 MBg1、MBg2 及 MBg3 之 ITS 序列彼此間具有 100% 相同度 (identity) (GenBank accession number HQ827770)，且與基因庫中 *B. gladioli* pv. *gladioli* 菌株 NCPPB1891 (GenBank accession number EF552070) 及 *B. gladioli* 菌株 ATCC10248 (GenBank accession number L28157) 的相似度達 97%。依 Schaad 等人 [17] 之方法分析菌株 MBg1、MBg2 及 MBg3 的生理生化特性，結果如表一所示。菌株 MBg1、MBg2 及 MBg3 爲革蘭氏陰性菌，此細菌可於 41℃ 下生長，但不能於 4℃ 或含 5% NaCl 之 NA 培養基上生長，且具有明膠 (gelatin)、脂質 (lipid)、幾丁質 (chitin)、酪蛋白 (casein) 等分子的分解能力與氧化酶 (oxidase)、觸酶 (catalase) 等酵素活性，與 *B. gladioli* 的特性相近。綜合上述脂肪酸圖譜分析、Biolog 鑑定系統、ITS 序列分析及各項生理生化測試結果，將分離自杏鮑菇之病原細菌鑑定爲 *B. gladioli*。

爲確認上述分離菌株的病原性，將菌株 MBg1、MBg2、MBg3 分別培養於 NA 平板培養基、30℃ 2 天，以無菌水懸浮後調整菌量爲 OD_{600} = 0.3 之細菌接種液 (濃度約爲 10^8 CFU/ml)，利用滲透注射法 (syringe infiltration) 接種於菸草葉片 (*Nicotiana tabacum* 臺煙 5 號品種)，將接種後的植物置於 30℃、12 小時光照處理之植物生長箱中，24 小時後即可在菸草葉片接種區域誘發壞疽病徵，但以無菌水接種的對照組則無病徵 (data not shown)。另以前述方法調製之 OD_{600} = 0.3 細菌接種液經 10 倍系列稀釋至 10^5 CFU/ml，每一稀釋菌液 (10^5、10^6、10^7、10^8 CFU/ml) 各取 200 μl 以穿刺法 (microtip-stab inoculation) 接種於外表無病徵之杏鮑菇蕈柄。將接種後的子實體置入高濕的塑膠盒中，於 16℃ 生長箱培養 6 天後，將接種之菇體自穿刺處剖開並觀察病徵發展情形。結果顯示菌株 MBg1、

表一、杏鮑菇細菌性腐敗病菌之生理生化特性

Table 1. Physiological and biochemical characteristics of the bacterial strains isolated from king oyster mushroom

Characteristics	Strains from		*B. gladioli*[3]
	King oyster mushroom[1]	Pineapple[2]	
KOH test	G (–)	G (–)	G (–)
Wrinkled colonies on NA	+[4]	+	–
Fluorescent pigment on KB	–	–	–
Diffusible, non-fluorescent pigments on KB	+	+	+
Diffusible pigments	+	+	+
Growth at 4°C	–	–	±
Growth at 41°C	+	+	+
Growth in 5% NaCl	–	+	–
Oxidase	+	+	V+
Catalase	+	+	+
Nitrate reduction	–	–	–
Arginine hydrolysis	–	–	–
Starch hydrolysis	–	–	–
Gelatin hydrolysis	+	+	+
Lipid hydrolysis	+	+	+
Pectate hydrolysis	–	–	–
Casein hydrolysis	+	+	+
Chitin hydrolysis	+	+	ND
Lecithinase	+	+	+

[1] The bacterial strains of king oyster mushroom bacterial decay disease used in the tests are MBg1, MBg2 and MBg3.
[2] The pathogens of pineapple fruit rot disease used in the tests are *B. gladioli* strains Bg3 and Bg5.
[3] Data are collected from different sources [2,10,13,17,21].
[4] +, positive; –, negative; V+, 21-79% positive; ±, strains variable; ND, not determined.

MBg2 及 MBg3 在接種濃度超過 10^7 CFU/ml 時即可在子實體接種處造成組織凹陷、擠壓後有菌泥溢出等病徵 (圖一、B與C)，與接種唐菖蒲首腐病菌 (*B. gladioli* pv. *gladioli*、簡稱 Bgg) 所造成的病徵相似 (圖一、C)，且於組織凹陷處皆可分離到與接種源相似的細菌，接種鳳梨果腐病菌 (*B. gladioli* 菌株 Bg3、Bg5) 則產生壞疽病徵 (圖一、C)，而接種無菌水的對照組則無病徵產生。進一步將杏鮑菇菌株 (MBg1、MBg2 及 MBg3)、唐菖蒲首腐病菌 (Bgg) 及鳳梨果腐病菌菌株 (Bg3 及 Bg5) 以針刺法 (needle-prick inoculation) 分別接種至株齡爲 2～3 葉之唐菖蒲葉片 (*Gladiolus hybridus* Hort. cv. Amsterdam and cv. Pink Giant)。針刺法乃利用消毒之 23 號注射針頭於唐菖蒲葉片上製造傷口，取 5 μl 依前述方法調製成之細菌懸浮液 (OD_{600} = 0.3) 或無菌水滴於傷口、使其風乾後，將全株植物以透明塑膠袋套袋保濕，再置入 30℃ 的生長箱，兩天後解開套袋、觀察並記錄病徵發展情形。結果顯示杏鮑菇菌株 MBg1、MBg2、MBg3、唐菖蒲首腐病菌 Bgg 及鳳梨果腐病菌菌株 Bg3、Bg5 等菌株皆可在唐菖蒲葉片上產生侷限型壞疽病徵，而接種無菌水之對照組則無產生病徵。再取 10 μl、OD_{600} = 0.3 之杏鮑菇菌株 MBg1、MBg2 及 MBg3、唐菖蒲首腐病菌及鳳梨果腐病菌菌株 Bg3、Bg5 的細菌懸浮液滴於洋蔥 (*Allium cepa*) 鱗片、或吸取 200 μl 之細菌懸浮液直接以穿刺法接種於經表面消毒之洋蔥鱗莖進行病原性測試，將接種後的洋蔥分別置入高濕培養皿 (鱗片) 與保鮮盒 (鱗莖) 中，於 30℃ 的生長箱培養兩天後觀察並記錄病徵發展情形。結果顯示杏鮑菇菌株 MBg1、MBg2、MBg3、唐菖蒲首腐病菌 Bgg 及鳳梨果腐病菌菌株 Bg3、Bg5 皆可於洋蔥鱗片產生組織凹陷的病徵，在洋蔥鱗莖穿刺接種處則產生褐化壞疽病徵。綜合以上病原性測試結果 (表二)，得知菌株 MBg1、MBg2 及 MBg3 對杏鮑菇具病原性，對唐菖蒲及洋蔥則具弱病原性，且其寄主範圍與文獻記錄之 *B. gladioli* pv. *agaricicola* [10] 相近。根據病原菌生理生化特性及其造成的病徵，本研究將 *B. gladioli* 在杏鮑菇上造成的病害定名爲杏鮑菇細菌性腐敗病，此爲 *B. gladioli* 在臺灣造成杏鮑菇細菌性腐敗病之首次報導。

本研究發現引起杏鮑菇細菌性腐敗病之病原菌爲 *B. gladioli*，與文獻中記載可危害杏鮑菇的病原細菌 *P. tolassii* [4] 及 *Pantoea* sp. [8] 不同。*B. gladioli* 寄主範圍相當廣泛，舉凡唐菖蒲 [11]、洋蔥 [16]、洋菇 [10]、小蒼蘭 [7]、石斛蘭 [7, 18]、蝴蝶蘭 [7]、水稻 [12, 19] 及鳳梨 [6] 等植物均有被此病原細菌感染的報導，且依其血清型及對寄主植物的感染能力不同，可將 *B. gladioli* 區分爲三個病原小種 (pathovar)，分別爲唐菖蒲病原小種 (pv. *gladioli*)、洋蔥病原小種 (pv. *alliicola*) 與洋菇病原小種 (pv. *agaricicola*) [10]。比較本研究分離之杏鮑菇細菌性腐敗病菌 *B. gladioli* 與文獻中洋菇細菌性軟腐病菌 *B. gladioli* pv. *agaricicola* [10] 的特性後，得知杏鮑菇細菌性腐敗病菌與洋菇細菌性軟腐病菌之脂肪酸組成及寄主

表二、杏鮑菇細菌性腐敗病菌之病原性測試

Table 2. Pathogenicity tests of *Burkholderia gladioli* on various hosts

Source / Host	Strains from[1]			*Burkholderia gladioli* pv.		
	King oyster mushroom	Pineapple	*Gladiolus hybridus*	*gladioli*[2]	*alliicola*[2]	*agaricicola*[2]
Rotting of tissue slices						
Mushroom slices	+[3]	+	ND	+	+	+
Onion slices	+	+	ND	+	+	±
Rotting of whole organs						
Mushroom	+	+	+	±	+	+
Onion bulbs	±	±	±	−	+	±
Necrosis and water soaking of leaves						
Gladiolus leaves	±	±	±	+	±	±

[1] The bacterial strains of king oyster mushroom bacterial decay disease used for pathogenicity tests are MBg1, MBg2 and MBg3, the pathogens of pineapple fruit rot disease are *Burkholderia gladioli* strains Bg3 and Bg5, and the strain that causes bacterial blight of *Gladiolus hybridus* is *B. gladioli* pv. *gladioli* strain Bgg. Host tissues and organs are inoculated with 10^8 CFU/ml and incubated at 30 ˚C, 2 days for symptom observation. The 3 strains of king oyster mushroom bacterial decay disease and the 2 strains of pineapple fruit rot disease elicit similar reactions on the testing hosts, which are summarized in the first and second columns of Table 2.

[2] Data are retrieved from Lincoln and Fermor (1991)[10] by inoculating *B. gladioli* pv. *gladioli* strain NCPPB1891, *B. gladioli* pv. *alliicola* stain NCPPB947, and *B. gladioli* pv. *agaricicola* strain NCPPB3580 to the listed plant tissues at 28 ˚C.

[3] Symptoms are scored at 2 days post inoculation (dpi) at 30 ˚C. −, no symptoms; +, symptoms are produced in 2 days; ±, restricted rot or necrosis at 2 dpi; ND, not determined.

範圍相近，但由於本研究未測試杏鮑菇細菌性腐敗病菌的血清型與其對洋菇、馬鈴薯、紅蔥頭、及小蒼蘭等寄主植物的病原性，故僅將杏鮑菇細菌性腐敗病菌命名爲 *B. gladioli*，其病原小種是否爲 pv. *agaricicola* 仍須進一步確認。

由於杏鮑菇易受紅蜘蛛或微生物等生物因子汙染，進而影響杏鮑菇之品質與產量，因此，杏鮑菇之菌種培養及栽培皆須在無菌條件下進行，栽培庫間首先須經過徹底清潔，且栽培介質必經過高壓滅菌處理，此外，杏鮑菇栽培期間之相對濕度常保持在 80% 左右，若栽植過程中栽培場內外的衛生管理不當，汙染源便可能經由空氣、水、栽培介質、設施、器具以及人員不當操作等方式傳播[14, 15]，造成杏鮑菇的感染。本研究在杏鮑菇栽培場發現之杏鮑菇細菌性腐敗病即可能是透過上述方式傳播，因此加強栽培庫間週遭環境及水源的衛生管理，應可達到預防此病害發生的目的。

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ABSTRACT

Lin, Y. T. [1], Lue, Y. S. [1, 2], Chang, Y. T. [1], and Deng, W. L. [1, 3] 2011. Bacterial decay of king oyster mushroom caused by *Burkholderia gladioli*. Plant Pathol. Bull. 19: 249-254. ([1] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan; [2] Plant Pathology Division, Agricultural Research Institute, Council of Agriculture, Wufeng, Taichung, Taiwan; [3] Corresponding author, E-mail: wdeng@dragon.nchu.edu.tw; Fax: +886-4-2287-7585)

In the year of 2008, caps and stalks of king oyster mushrooms exhibiting decayed and sunken symptoms were found in a mushroom farm in Daili, Taichung. Three bacterial strains, MBg1, MBg2, and MBg3, isolated from the decay tissues were assayed physiologically and biochemically to show that they are gram-negative, non-fluorescent bacterium which can grow at the temperature up to 41°C, produce yellow pigment on nutrient agar and King's B media, catalyze gelatin, lipid, chitin, and casein on culture media, and have oxidase and catalase activities. The MBg strains were characterized using fatty acid methyl ester analysis (Agilent Technologies, Santa Clara, CA) and Sherlock® Microbial Identification System (Microbial Identification Inc., Newark, DE), and each strain was identified as *Burkholderia gladioli* with a similarity greater than 0.83. In addition, the Biolog system (Biolog, Hayward, CA) and sequence identity comparisons of 16S-23S intergenic transcribed spacer (ITS) were performed to confirm that the 3 bacterial strains isolated from king oyster mushroom are *B. gladioli* based on a similarity of 0.58 with Biolog and 99% sequence identity for 16S-23S ITS. Koch's postulates were fulfilled by inoculating the suspensions of MBg stains at 10^8 CFU/ml to the stalks of disease-free king oyster mushroom, and bacteria showing morphological and biochemical similarities to the ones used for inoculation were isolated from the inoculated mushrooms at 6 days post inoculation. This is the first report that *B. gladioli* can infect king oyster mushroom to elicit bacterial decay disease in Taiwan.

Keywords: Bacterial decay of king oyster mushroom, *Burkholderia gladioli*, fatty acid methyl ester analysis, 16S-23S internal transcribed spacer sequence