

## 論文

# 台灣溫室作業環境生物氣膠分佈特性

楊心豪<sup>1</sup> 黃筱茜<sup>1</sup> 洪柏宸<sup>2</sup> 莊啓佑<sup>3</sup> 方煒<sup>3</sup>

<sup>1</sup> 稻江科技暨管理學院通識中心

<sup>2</sup> 勞動部勞動及職業安全衛生研究所

<sup>3</sup> 國立台灣大學生物產業機電工程學系

## 摘要

近本研究主要針對溫室作業場所進行空氣中細菌與真菌之調查，以瞭解目前台灣之溫室作業場所中生物性危害之程度。研究中選取3間家溫室作業場所進行採樣分析，細菌與真菌生物氣膠分佈是利用衝擊式(impactor)生物氣膠採樣器進行採樣，採樣分為上午、中午與下午三時段進行採樣。生物氣膠採集後之真菌與細菌樣本則進一步將其純化培養，再予以分子生物鑑定方式進行菌種鑑定。研究結果發現，平均細菌生物氣膠濃度達到最高 $1,674 \pm 296$ CFU/m<sup>3</sup>，真菌生物氣膠最高可達到 $6,432 \pm 981$ CFU/m<sup>3</sup>。菌種鑑定結果顯示，真菌有*Trichoderma* spp.、*Penicillium* spp.、*Aspergillus* spp.、*Acremonium* spp.、*Meira* spp.。細菌則有*Bacillus* spp.、*Planobacterium* spp.、*Chryseobacterium* spp.、*Jeotgalicoccus* spp.、*Staphylococcus* spp.、*Nocardiopsis* spp.、*Agromyces* spp.、*Micrococcus* spp.。

**關鍵字：**溫室作業環境、生物氣膠、細菌、真菌、分佈

---

民國 101 年 11 月 12 日投稿，民國 103 年 12 月 8 日修改，民國 104 年 1 月 31 日接受。

通訊作者：楊心豪，稻江科技暨管理學院通識教育中心，61363嘉義縣朴子市學府路2段51號，

電子郵件信箱：shinhaoyang@ntu.edu.tw。

## 前言

溫室是目前台灣農業中針對高經濟附加價值之作物主要使用之種植環境，為台灣設施農業中主要的部分。然溫室設施內之園藝作物栽培過程中，由於其位處於室內空間，通風換氣效果不如室外，濕氣容易累積且內部又具有土壤、植株、水源等微生物可能寄居生長之來源，故生物氣膠容易繁衍與累積。此些農業相關微生物則藉由人員作業、植物搬運、空氣通風、用灌溉水等途徑造成散佈，溫室作業人員因作業過程中會直接接觸生物氣膠，特別容易暴露於微生物危害之中，目前雖然並無因為接觸溫室中之微生物造成感染危害之實際案例，但是微生物高度暴露所造成之呼吸道症狀在近年來相當受到重視。

過去國外文獻中對於溫室作業環境內生物氣膠濃度分佈與健康效應均有探討，Radon et al.(2002)研究發現在西班牙37家溫室中活性真菌濃度為 $8.3 \times 10^4$ CFU/m<sup>3</sup>，活性細菌濃度為 $4.1 \times 10^4$ CFU/m<sup>3</sup>[1]。Mons(2004)則發現在採樣之溫室中活性真菌濃度為1,700-11,000 CFU/m<sup>3</sup>，活性細菌濃度為770-5,200 CFU/m<sup>3</sup>。細菌內毒素濃度為0.17-0.89EU/m<sup>3</sup>[2]。Hansen et al. (2010)針對種植蕃茄之溫室進行生物氣膠量測，其中空氣中總細菌濃度 $9.6 \times 10^2$ - $3.4 \times 10^4$ CFU/m<sup>3</sup>與總真菌濃度 $2.6 \times 10^4$ - $3.7 \times 10^5$ CFU/m<sup>3</sup>[3]。Adhikari et al. (2011)發現花卉溫室中可呼吸性細菌及真菌濃度在 $10^2$ - $10^5$ CFU/m<sup>3</sup>間，細菌內毒素則在 $10^1$ - $10^3$ EU/m<sup>3</sup>間[4]。

文獻指出統計了多個花卉以及食用作物溫室所屬共4,108個勞工健康狀況後，發現作業人員可能具有皮膚搔癢、氣喘、過敏性支氣管炎及皮膚炎等症狀之風險[5]。文獻亦指出溫室環境勞工中因等*Cladosporium*、*Penicillium*、

*Aspergillus*與*Alternaria*等真菌而產生呼吸道過敏症狀者佔了30%，其中更有20%之勞工發現有職業性氣喘之狀況[2,6]。美國職業安全衛生研究所NIOSH於1995年針對職業衛生之普查結果則發現，溫室環境工作人員發生上呼吸道及下呼吸道症狀之比率，為所有農業作業環境工作人員之首位[7]。

由以上文獻顯示，在國外的調查中家溫室作業環境生物氣膠濃度相當之高，同時也對作業人員健康造成危害，而目前台灣對於溫室作業環境生物氣膠分佈之調查卻是較為缺乏的，因此本研究即是針對溫室作業環境生物氣膠分佈進行調查，在本研究調查過程中，除針對溫室作業環境細菌與真菌生物氣膠濃度分佈進行調查，也進行菌種鑑定以瞭解此類作業環境中主要環境菌種為何，另採樣過程中也會針對作業環境設施之環境特性進行採樣，方可對溫室作業環境之生物氣膠分佈以及其影響因子有明確判斷。

## 研究方法

### 1. 溫室採樣規劃

台灣目前在高經濟價值之作物栽培溫室設施中，較為常見為常荷蘭Venlo型溫室、鋼骨強化鋁管溫室及加強型鋁管塑膠布溫室等類型。不過無論是何種溫室，外觀及架構均是維持其基本造型與封閉環境，主要影響生物氣膠分佈仍是在於其溫度、濕度、風速、所使用之環境控制設備以及引入之外氣來源等因素上。本研究選取三家溫室進行採樣，為了代表性研究中選取之溫室為荷蘭Venlo型溫室與加強型鋁管塑膠布溫室；在作物分類上，均是高經濟附加價值作物溫室，其中包含蘭花栽培溫室以及金線連栽培溫室，採樣時間在於2011年6月。

溫室設施內部之採樣位置，參考我國環保署NIEA E301.14C「室內空氣中總細菌檢測方法」及NIEA E401.14C「室內空氣中總真菌數檢測方法」之規範，採樣高度則為距離地面80-100cm高（考量溫室作業人員均為彎腰或坐姿進行植物栽培及養護作業），以模擬人類呼吸帶之暴露。採樣位置與數目主要依據現場大小、作業人員工作內容以及作業環境污染來源進行設置。此外，本研究在每一個採樣點，亦同步紀錄採樣工作進行時之溫度、濕度以及風速等室內微環境條件，以利進行室內污染來源推估以及人員暴露評估。

圖1-圖3為本研究計畫所選取之溫室平面圖，三家溫室場所均屬於室內作業場所。每一溫室均進行三重複採樣，並同時於早上、中午與下午進行採樣，以比較不同時段對於作業環境之生物氣膠分佈是否具有差異。每一溫室均有散熱水簾以及抽氣風扇等裝置。

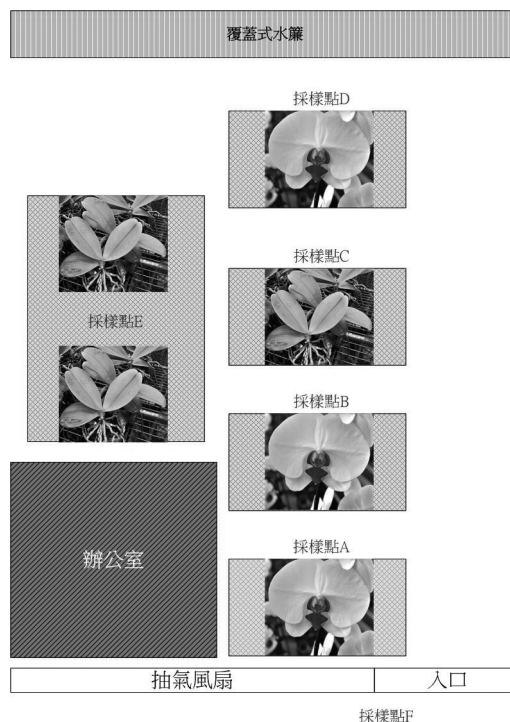


圖3 溫室作業環境3平面圖

## 2. 活性生物氣膠採樣

本研究之細菌與真菌生物氣膠採樣及監測工作，是使用衝擊式生物氣膠採樣器(Biostage Simple-stage Vcable Cascade Impactor, SKC Inc., USA)，此採樣器上有400孔，孔徑為0.25 mm，採樣時以充電電池啟動內置馬達抽取空氣，當氣流轉變方向時，細菌及真菌因慣性被收集到培養基上，採樣流量是28.3L/min，採樣前後該機器本身會自行校正其流量，使用之採樣介質為倒入27mL培養基之直徑90mm可拋棄式塑膠培養皿。本研究中採用2種培養基，Malt Extract Agar(MEA)及Trypticase Soy Agar(TSA)。其中MEA為美國工業衛生師協會(American Conference of Government Industrial Hygienists, ACGIH)推薦使用的廣效性培養基[8-9]，可提供大部份的真菌生長，而TSA則用以收集培養空氣中之細菌。每個採樣點進行兩次採樣，分別是在早上、中午以及下午進行採

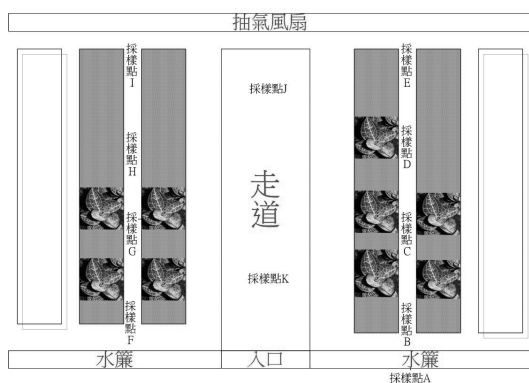


圖1 溫室作業環境1平面圖

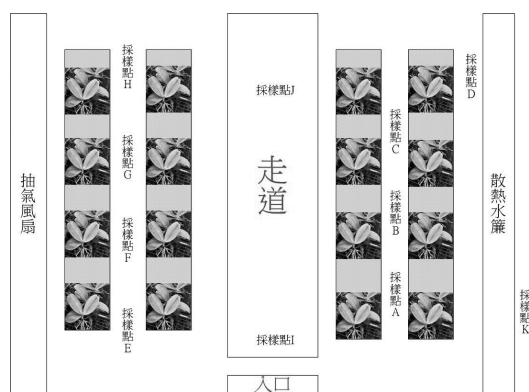


圖2 溫室作業環境2平面圖

樣，除在每個採樣點均附以重複試驗進行。樣後將培養基放入恆溫培養箱中培養。經前測後，目前採樣時間是定為30秒鐘之採樣時間進行採集，以避免出現造成無法計數(too many to count, TNTC)之情形。

針對採集培養細菌使用之TSA及真菌使用之MEA培養基之配置方法，將按照兩種培養基之標準配方秤取所需之培養基成分加上適量之去離子水均勻混合後，放入加壓滅菌釜以121°C高溫進行滅菌20分鐘。滅完菌後，待培養基冷卻之55-60°C，分裝至27ml的培養基至培養皿(90×15mm)中，待其凝固後儲存於4°C冰箱中備用。

TSA細菌培養基必須於 $30 \pm 1^\circ\text{C}$ 培養 $48 \pm 2$ 小時後計數菌落數，而MEA真菌之培養則必須於 $25 \pm 1^\circ\text{C}$ 培養箱內培養 $4 \pm 1$ 天後計數。樣本均以菌落數校正表(positive hole conversion table)校正菌落數，而後根據校正菌落數、採樣流量及採樣時間等參數，依照下列公式回推為空氣中濃度即可推估溫室作業環境中生物氣膠之濃度。根據環保署標準方法，最小偵測極限則是以小於一個CFU做為估算依據，本研究採樣時間為10秒，因此下界為 $< 212\text{CFU}/\text{m}^3$ 。

$$\text{濃度}(\text{CFU}/\text{m}^3) = \text{校正後菌落數}(\text{CFU}) / [28.3(\text{L}/\text{min}) \times t(\text{min}) \times 10^{-3}(\text{m}^3/\text{L})] \dots\dots\dots (1)$$

### 3. 菌種鑑定

為進一步瞭解實際採集之活性細菌與真菌菌落之品種，根據過去文獻[10-11]指出，先進行真菌與細菌生物氣膠之採樣，採樣後進行純化，再以分子生物鑑定方式進行菌種鑑定，因此本研究在實際細菌與真菌採樣培養後，細菌部分初步先經分離純化後將型態相同者進行分類，再進一步則是以革蘭氏染色法初步進行分

子生物菌種鑑定。真菌則是初步先將型態相同者進行分離純化及分類，再轉殖純化後進行分子生物菌種鑑定，菌種鑑定中，細菌是利用抽取DNA進行16S rDNA比對鑑定，另真菌則是以抽取DNA進行18S rDNA比對鑑定。本研究鑑定主要鑑別至微生物之屬。

### 4. 環境特性偵測

本研究在採樣過程中，對於作業環境之特性也同時進行監測，分別利用風速計(PROVA AVM-03/AVM-01)量測家禽屠宰作業環境之風速，以及Q-trak (Model 7565, TSI Inc., USA) 量測作業環境之溫度與相對濕度。

### 5. 統計檢定

整體實驗數據檢定部分，由於生物氣膠一般是右偏分佈，因此採用對應的檢定方法為無母數分析方法，因此在實驗數據檢定方法為下，不同作業區域、作業時段生物氣膠濃度之差異分析是以Kruskal-Wallis Test進行顯著性差異分析；室內外生物氣膠之差異是以Wilcoxon Signed-Rank Test進行顯著性差異分析；環境條件對於生物氣膠分佈之影響是利用Spearman correlation coefficient進行分析。

## 結果與討論

### 1. 溫室作業場所1生物氣膠之分佈

本研究選擇之溫室作業場所1為金線連溫室，大小約為高4公尺、長22公尺、寬10公尺，場1作業環境平均換氣率為0.9(1/hr)，換氣率主要是利用換氣風扇之換氣量來進行估算，當風扇啟動後風扇之風速大約為4.5m/s，但其開啟的方式並不固定，而是由其設定的感溫裝置評估其啟動時機。針對溫室作業場所1共規

劃11個採樣點，除A點為室外採樣點，而其餘都為室內採樣點。

圖4與5為在場1針對11個採樣點在三重複的採樣下細菌及真菌類生物氣膠濃度分佈統計圖，表1則是採樣過程中環境條件之彙整表。結果顯示溫室作業環境場1場外採樣點細菌生物氣膠濃度在 $254 \pm 43$ - $495 \pm 104$  CFU/m<sup>3</sup>間，在場內採樣點部分細菌生物氣膠濃度在 $212$   $33$ - $1,413 \pm 324$  CFU/m<sup>3</sup>間。最高濃度出現於採樣採樣點D之中午時段，最低濃度出現於採樣點C之下午時段。在真菌生物氣膠部分，結果顯示場外採樣點真菌生物氣膠濃度在 $495 \pm 201$ - $777 \pm 211$  CFU/m<sup>3</sup>間，在場內真菌生物氣膠濃度在 $565 \pm 143$ - $3463 \pm 365$  CFU/m<sup>3</sup>間。最高濃度出現於採樣採樣點I早上時段，最低濃度出現於採樣點E 下午時段。

整體而言，以Wilcoxon Signed Rank test分析結果顯示，場內細菌與生物氣膠濃度是高於場外細菌生物氣膠濃度( $p < 0.05$ ,  $n = 99$ )；利用Kruskal-Wallis Test分析場內不同作業時段之細菌生物氣膠濃度，結果顯示早上作業中細菌生物氣膠濃度也高於另外兩個時段( $p < 0.05$ ,  $n = 99$ )；利用Kruskal-Wallis Test分析場內不同採樣點細菌生物氣膠濃度，顯示並無顯著差異( $p > 0.05$ ,  $n = 99$ )，若單獨比較種植床架區域與抽氣口區域細菌生物氣膠量也無顯著差異。在真菌部分整體生物氣膠特性與細菌生物氣膠

顯示之結果均相同。利用Spearman correlation coefficient進行分析環境條件（如表1所示）對於細菌與真菌生物氣膠濃度之相關性分析，由分析結果可知，環境因子對細菌與真菌生物氣膠之相關性分析皆無顯著性。

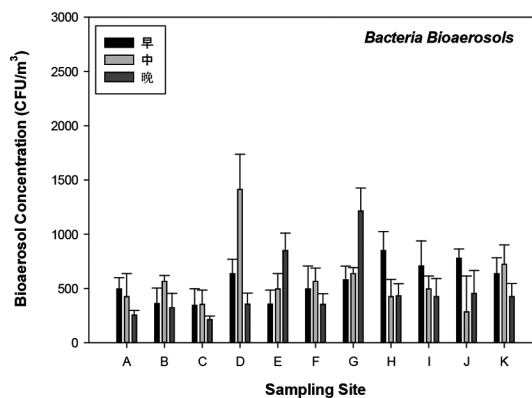


圖4 溫室作業場所1細菌生物氣膠濃度分佈

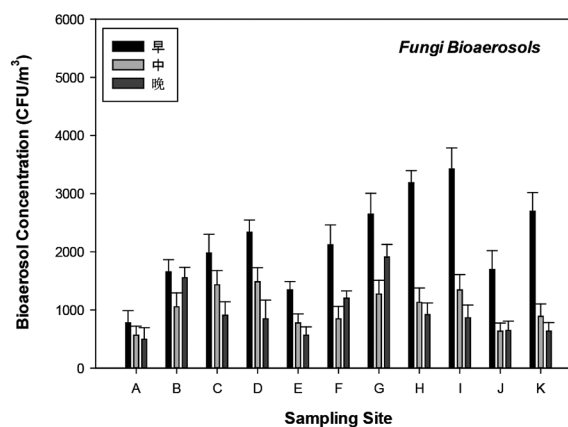


圖5 溫室作業場所1真菌生物氣膠濃度分佈

表1 溫室作業場所1採樣時環境條件

採樣點	溫度(°C)			濕度(%)			風速(cm/s)		
	上午	中午	下午	上午	中午	下午	上午	中午	下午
A (室外)	29.1	33.0	29.2	80	67	80	0.11	0.15	0.13
B	29.1	33.1	29.0	81	68	80	0.15	0.18	0.18
C	29.0	33.0	29.1	80	67	81	0.12	0.16	0.15
D	29.1	33.2	29.0	80	67	80	0.14	0.19	0.23
E	29.2	33.0	28.9	79	68	81	0.14	0.17	0.15
F	29.0	33.1	29.0	80	69	82	0.09	0.19	0.18
G	29.0	33.0	29.0	81	68	80	0.13	0.20	0.14
H	29.1	33.1	29.1	78	67	80	0.14	0.18	0.11
I	29.0	33.0	29.2	79	67	80	0.11	0.19	0.18
J	29.0	33.1	29.0	80	68	81	0.15	0.21	0.18
K	30.1	33.5	29.5	77	66	79	0.08	0.09	0.15

\*n=99 (11個採樣點，量測3種環境因子，分別量測上午、中午、下午)

在菌種鑑定的部分，結果顯示真菌主要為 *Penicillium* spp.、*Aspergillus* spp.、*Acremonium* spp.、*Meira* spp.。在細菌部分主要包含 *Nocardiosis* spp.、*Agromyces* spp.、*Bacillus* spp.、*Micrococcus* spp.、*Staphylococcus* spp.。

## 2. 溫室作業場所2生物氣膠之分佈

溫室作業場所2為蘭花溫室，作業環境大小約為高4.5公尺、長30公尺、寬12公尺，場2作業環境平均換氣率為1.2(1/hr)，換氣率主要是利用換氣風扇之換氣量來進行估算，當風扇啟動後風扇之風速大約為6m/s，但其開啟的方式並不固定，而是由其設定的感溫裝置評估其啟動時機，作業環境之風向是由水簾處往抽氣風扇處移動。針對溫室作業場所1共規劃11個採樣點，除K點為室外採樣點，而其餘都為室內採樣點。

圖6與7為在作業場所2中，各採樣點之細菌及真菌類生物氣膠濃度分佈統計圖，表2則是採樣過程中環境條件之彙整表。結果顯示溫室作業環境場1場外採樣點細菌生物氣膠濃度在 $431 \pm 105$ - $843 \pm 113$  CFU/m<sup>3</sup>間，在場內採樣點部分細菌生物氣膠濃度在 $212 \pm 54$ - $1,674 \pm 296$  CFU/m<sup>3</sup>間。最高濃度出現於採樣採樣點J之下午時段，最低濃度出現於採

樣點C之下午時段。在真菌生物氣膠部分，場外採樣點真菌生物氣膠濃度在 $1,145 \pm 211$ - $3,932 \pm 536$  CFU/m<sup>3</sup>間，場內真菌生物氣膠濃度在 $1,214 \pm 352$ - $6,432 \pm 981$  CFU/m<sup>3</sup>間。最高濃度出現於採樣採樣點F中午時段，最低濃度出現於採樣點A下午時段。

整體而言，以Wilcoxon Signed Rank test分析結果顯示，場內細菌與生物氣膠濃度是高於場外細菌生物氣膠濃度( $p < 0.05$ , n=99)；利用Kruskal-Wallis Test分析場內不同作業時段之細菌生物氣膠濃度，結果顯示不同作業時間並無顯著差異( $p > 0.05$ , n=99)；利用Kruskal-Wallis Test分析場內不同採樣點細菌生物氣膠濃度，顯示並無顯著差異( $p > 0.05$ , n=99)，若單獨比較種植床架區域與抽氣口區域細菌生物氣膠量也無顯著差異。

以Wilcoxon Signed Rank test分析結果顯示，場內真菌與生物氣膠濃度是高於場外真菌生物氣膠濃度( $p < 0.05$ , n=99)；利用Kruskal-Wallis Test分析場內不同作業時段之細菌生物氣膠濃度，結果顯示中午作業時段真菌生物氣膠明顯高於其他兩時段( $p < 0.05$ , n=99)；利用Kruskal-Wallis Test分析場內不同採樣點真菌生物氣膠濃度，顯示並無顯著差異( $p < 0.05$ , n=99)。

利用Spearman correlation coefficient進行分析環境條件（如表2所示）對於細菌與真菌生物氣膠濃度之相關性分析，由分析結果可知，環境因子對細菌與真菌生物氣膠之相關性分析皆無顯著性。

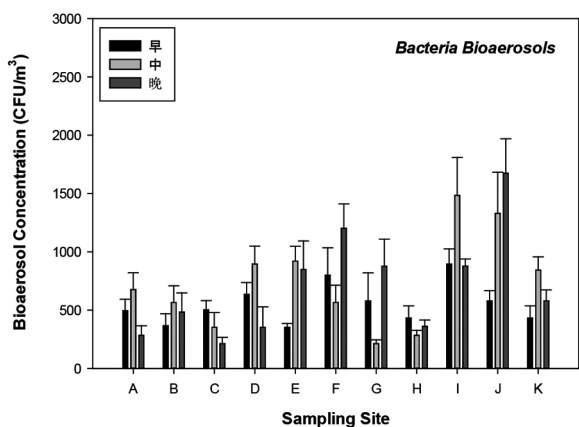


圖6 溫室作業場所2細菌生物氣膠濃度分佈

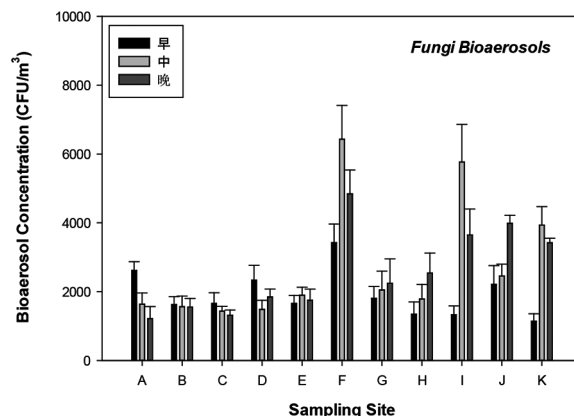


圖7 溫室作業場所2真菌生物氣膠濃度分佈

表2 溫室作業場所2採樣時環境條件

採樣點	溫度(°C)			濕度(%)			風速(cm/s)		
	上午	中午	下午	上午	中午	下午	上午	中午	下午
A	30.0	32.1	29.3	81	83	87	0.13	0.15	0.22
B	30.0	32.1	29.4	81	84	86	0.15	0.16	0.14
C	30.1	32.2	29.3	82	84	86	0.16	0.16	0.24
D	30.2	32.3	29.5	81	83	87	0.15	0.18	0.21
E	30.1	32.1	29.4	82	83	87	0.12	0.21	0.13
F	30.1	32.1	29.4	82	83	88	0.11	0.11	0.15
G	29.9	32.2	29.5	81	85	87	0.13	0.14	0.16
H	30.0	32.0	29.4	81	83	87	0.13	0.15	0.22
I	30.1	32.1	29.4	81	83	88	0.14	0.16	0.16
J	30.2	32.1	29.3	80	83	88	0.15	0.19	0.14
K (室外)	31.1	33.3	30.1	75	76	85	0.18	0.17	0.19

\*n=99 (11個採樣點，量測3種環境因子，分別量測上午、中午、下午)

鑑定出的結果中，真菌主要為*Trichoderma* spp.、*Penicillium* spp.、*Aspergillus* spp.、*Acremonium* spp.。在細菌部分主要包含*Bacillus* spp.、*Planobacterium* spp.、*Chryseobacterium* spp.、*Jeotgalicoccus* sp.、*Staphylococcus* spp.、*Staphylococcus* spp.。

### 3. 溫室作業場所3生物氣膠之分佈

溫室作業場所3為蘭花溫室，作業環境大小約為高4公尺、長15公尺、寬10公尺，場3作

業環境無換氣（全天冷氣），實場環境內風速為0.05-0.6 m/s。針對溫室作業場所3共規劃6個採樣點，除F點為室外採樣點，而其餘都為室內採樣點。

圖8與9為在場3針對6個採樣點在三重複的採樣下細菌及真菌類生物氣膠濃度分佈統計圖，表3則是採樣過程中環境條件之彙整表。結果顯示溫室作業環境場3場外採樣點細菌生物氣膠濃度在 $283 \pm 54$ - $353 \pm 21$  CFU/m<sup>3</sup>間，在場內採樣點部分細菌生物氣膠濃度在 $283 \pm 54$ -

1,272 ± 209 CFU/m<sup>3</sup>間。最高濃度出現於採樣採樣點E之中午時段，最低濃度出現於採樣點A之中午時段。

整體而言，以Wilcoxon Signed Rank test分析結果顯示，場內與場外細菌生物氣膠濃度無顯著差異( $p>0.05$ ,  $n=54$ )；利用Kruskal-Wallis Test分析場內不同作業時段之細菌生物氣膠濃度，結果顯示不同時段細菌生物氣膠濃度無顯著差異( $p>0.05$ ,  $n=54$ )；利用Kruskal-Wallis Test分析場內不同採樣點細菌生物氣膠濃度，顯示並無顯著差異( $p>0.05$ ,  $n=54$ )。

場3中場外採樣點真菌生物氣膠濃度在1,032 ± 231-1,564 ± 325 CFU/m<sup>3</sup>間，在場內真菌生物氣膠濃度在1,019 ± 201-2,987 ± 312 CFU/m<sup>3</sup>間。最高濃度出現於採樣採樣點B下午時段，最低濃度出現於採樣點C上午時段。

以Wilcoxon Signed Rank test分析結果顯示，場內真菌與生物氣膠濃度是高於場外真菌生物氣膠濃度( $p<0.05$ ,  $n=54$ )；利用Kruskal-Wallis Test分析場內不同作業時段之細菌生物氣膠濃度，結果顯示下午作業時段真菌生物氣膠明顯高於其它兩時段( $p<0.05$ ,  $n=54$ )；利用Kruskal-Wallis Test分析場內不同採樣點真菌生物氣膠濃度，顯示並無顯著差異( $p<0.05$ ,  $n=54$ )。

利用Spearman correlation coefficient進行分析環境條件（如表3所示）對於細菌與真菌生

物氣膠濃度之相關性分析，由分析結果可知，相對濕度對於真菌生物氣膠濃度有顯著相關性（相關係數 $r=0.412$ ,  $p<0.05$ ），由相關係數可知相對濕度與真菌生物氣膠濃度是屬中度相關，而其他環境因子對細菌與真菌生物氣膠之相關性分析皆無顯著性。

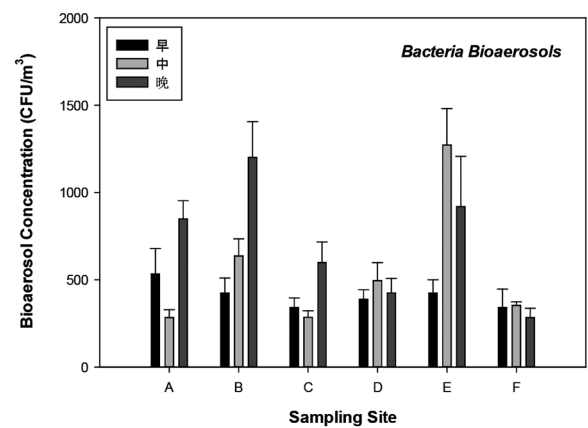


圖8 溫室作業場所3細菌生物氣膠濃度分佈

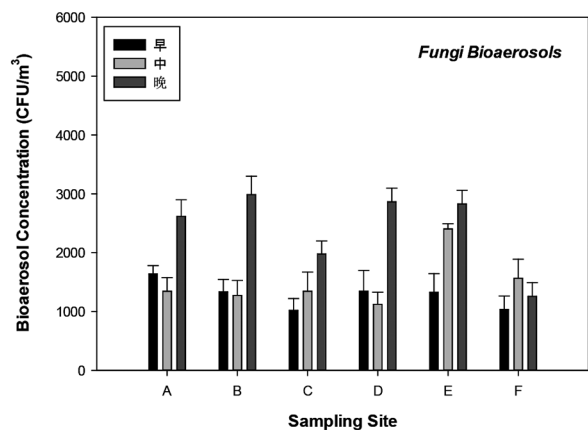


圖9 溫室作業場所3細菌生物氣膠濃度分佈

表3 溫室作業場所3採樣時環境條件

採樣點	溫度(°C)			濕度(%)			風速(cm/s)		
	上午	中午	下午	上午	中午	下午	上午	中午	下午
A	29.1	28.0	22.1	60	62	77	0.03	0.05	0.05
B	29.1	28.1	22.2	61	62	78	0.04	0.04	0.06
C	29.2	28.2	22.3	60	61	77	0.03	0.03	0.05
D	29.3	28.1	22.2	62	61	77	0.03	0.04	0.06
E	29.1	28.0	22.1	60	61	77	0.03	0.05	0.06
F (室外)	30.1	30.5	28.8	62	66	68	0.11	0.12	0.12

\*n=54 (6個採樣點，量測3種環境因子，分別量測上午、中午、下午)



菌種鑑定結果顯示，真菌生物氣膠主要為 *Penicillium* spp.、*Aspergillus* spp.。在細菌生物氣膠部分主要為 *Bacillus* spp.、*Planobacterium* spp.、*Chryseobacterium* spp.、*Jeotgalicoccus* spp.、*Staphylococcus* spp.。

將3家溫室作業場所之採樣結果與文獻之結果[2-4]相比較，3場家作業場所不論在細菌或是真菌生物氣膠濃度均是低於文獻值的，顯示台灣本土細菌與真菌生物氣膠濃度是較低的。與文獻[2,6]比較，結果均發現主要菌種均有 *Penicillium* spp. 與 *Aspergillus* spp.。就濃度而言，作業場所2與3均是蘭花溫室，其中真菌生物氣膠濃度均明顯高於場所1，而場所則是屬於金線連溫室。另就環境因子的影響來看，在3家溫室作業場所之分析結果來看，僅有相對濕度對於真菌生物氣膠濃度有出現顯著相關性，雖然僅是中度相關，不過也可以讓我們瞭解相對濕度過高對於生物氣膠濃度是有影響的。

菌種部分，在真菌有 *Trichoderma* spp.、*Penicillium* spp.、*Aspergillus* spp.、*Acremonium* spp.、*Meira* spp.。其中 *Trichoderma* spp. 為木黴菌無文獻指出對於人體健康有影響；*Aspergillus* spp. 為伺機性感染的菌屬，有過敏性麴菌症，這些症狀包括發燒，咳嗽，胸痛，呼吸困難；*Penicillium* spp. 對人體健康則無顯著影響；*Acremonium* spp. 為伺機性病原體；*Meira* spp. 為黴菌一種，目前無文獻指出對人體健康有影響。

細菌則有 *Bacillus* spp.、*Planobacterium* spp.、*Chryseobacterium* spp.、*Jeotgalicoccus* spp.、*Staphylococcus* spp.、*Nocardiopsis* spp.、*Agromyces* spp.、*Micrococcus* spp.。其中 *Bacillus* spp. 為芽孢桿菌，部分種會引起，導致食物中毒類如腸胃炎，眼部感染，血管內導管相

關敗血症等；*Planobacterium* spp. 為桿狀細菌菌株；*Chryseobacterium* spp. 為金黃桿菌屬；*Jeotgalicoccus* spp. 嗜鹽鹹海鮮球菌；*Staphylococcus* spp. 為葡萄球菌屬，常由人類傷口進入宿主感染，會引起發炎及化膿；*Nocardiopsis* spp. 為擬諾卡氏菌屬，部分菌種會引起肺部感染或造成皮下組織腫脹；*Agromyces* spp. 屬放線菌屬，無文獻顯示對人類健康有影響；*Micrococcus* spp. 為微球菌屬，無文獻顯示對人類健康有影響。

## 結論

溫室作業場所內，平均細菌生物氣膠濃度達到最高  $1,674 \pm 296$  CFU/m<sup>3</sup>，真菌生物氣膠最高可達到  $6,432 \pm 981$  CFU/m<sup>3</sup>。菌種鑑定結果顯示，真菌有 *Trichoderma* spp.、*Penicillium* spp.、*Aspergillus* spp.、*Acremonium* spp.、*Meira* spp.。細菌則有 *Bacillus* spp.、*Planobacterium* spp.、*Chryseobacterium* spp.、*Jeotgalicoccus* spp.、*Staphylococcus* spp.、*Nocardiopsis* spp.、*Agromyces* spp.、*Micrococcus* spp.。整體而言，本研究中本土溫室作業場所之細菌與真菌生物氣膠濃度部分則是低於國外文獻，菌種特性部分也是相符。根據採樣分析結果，就目前通風條件下，各採樣點之生物氣膠濃度仍是偏高，顯示在增加換氣以降低改善作業環境細菌及真菌生物氣膠濃度。

## 誌謝

本研究承蒙勞動部勞動及職業安全衛生研究所100年度研究計畫(IOSH100-H314)經費支持，謹此敬表謝忱。

## 參考文獻

[1] Radon K, Danuser B, Iversen M, Monso E,

- Weber C, Hartung J, Donham KJ, Palmgren U, Nowak D. Air contaminants in different European farming environments. *Annals of Agricultural and Environmental Medicine* 2002; 9: 41-8.
- [2] Mons E. Occupational asthma in greenhouse workers. *Current opinion in pulmonary medicine* 2004; 10: 147-50.
- [3] Hansen VM, Winding A, Madsen AM. Exposure to bioaerosols during the growth season of tomatoes in an organic greenhouse using Supresivit (*Trichoderma harzianum*) and Mycostop (*Streptomyces griseoviridis*). *Applied and environmental microbiology* 2010; 76: 5874-81.
- [4] Adhikari A, Gupta J, Wilkins JR, Olds RL, Indugula R, Cho KJ, Li C, Yermakov M. Airborne microorganisms, endotoxin, and (1→3)- $\beta$ -D-glucan exposure in greenhouses and assessment of respiratory symptoms among workers. *Annals of occupational hygiene* 2011; 55: 272-85.
- [5] Illing HPA. Is working in greenhouses healthy? Evidence concerning the toxic risks that might affect greenhouse workers. *Occupational medicine* 1997; 47: 281-93.
- [6] Monsó E, Magarolas R, Badorrey I, Radon K, Nowak D, Morera J. Occupational asthma in greenhouse flower and ornamental plant growers. *American journal of respiratory and critical care medicine* 2002; 165: 954-60.
- [7] Wilk V, Holden R. *New Directions in the Surveillance of Hired Farm Worker Health and Occupational Safety*. Cincinnati, OH: National Institute for Occupational Safety and Health; 1999.
- [8] Thorne PS, Kiekhaefer MS, Whitten P, Donham KJ. Comparison of bioaerosol sampling methods in barns housing swine. *Applied and environmental microbiology* 1992; 58: 2543-51.
- [9] Macher JM, Chatigny MA, Burge HA. Air sampling instruments for evaluation of atmospheric contaminants: Sampling airborne microorganisms and aeroallergens. *ACGIH* 1995, Cincinnati, Ohio. 589-617.
- [10] Acinas SG, Sarma-Rupavtarm R, Klepac-Ceraj V, Polz MF. PCR-induced sequence artifacts and bias: insights from comparison of two 16S rRNA clone libraries constructed from the same sample. *Applied and Environmental Microbiology* 2005; 71: 8966-9.
- [11] Chandler DP. Redefining relativity: quantitative PCR at low template concentrations for industrial and environmental microbiology. *Journal of Industrial Microbiology and Biotechnology* 1998; 21: 128-40.

Research Articles

# The Characteristics of Bacteria and Fungi Bioaerosols Distribution in Taiwan Greenhouses

Shinhao Yang<sup>1</sup> Hsiao-Chien Huang<sup>1</sup> Po-Chen Hung<sup>2</sup>  
Chi-Yu Chuang<sup>3</sup> Wei Fang<sup>3</sup>

<sup>1</sup> Center for General Education, Toko University, Toko University

<sup>2</sup> Institute of Occupational Safety and Health, Occupational Hygiene Division

<sup>3</sup> Department of Bio-Industrial Mechatronics Engineering, National Taiwan University

## Abstract

This study aims to investigate the airborne microorganisms and bacteria endotoxins in the greenhouse, for understanding biological hazards in Taiwan greenhouse. Three greenhouses were selected as the testing subjects. The bacteria and fungi bioaerosol distributions were sampling by impactor bioaerosol sampler. The species of bioaerosol samples in the operating workplace were finally purified, cultured, and further identified by molecular biology.

The results showed that in the greenhouses, the highest average bacteria bioaerosol concentrations was in the range of  $1674 \pm 296$  CFU/m<sup>3</sup>, and the highest average fungi bioaerosols concentration was about  $6,432 \pm 981$  CFU/m<sup>3</sup>. According to the identified of molecular biology in the greenhouses, bacteria bioaerosols were *Bacillus* spp., *Planobacterium* spp., *Chryseobacterium* spp., *Jeotgalicoccus* spp., *Staphylococcus* spp., *Nocardiopsis* spp., *Agromyces* spp., and *Micrococcus* spp.. The fungi bioaerosols were *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., *Acremonium* spp. and *Meira* spp.

**Keywords:** Bioaerosols, Greenhouse, Bacteria, Fungi, Distribution

---

Accepted 31 January, 2015

Correspondence to: Shinhao Yang, Associate Professor, Center for General Education, Toko University, No.51, Sec.2, Xuefu Rd., Puzi City, Chiayi County 61363, Taiwan(R.O.C), E-mail: shinhaoyang@ntu.edu.tw

## Introduction

Greenhouses are major agricultural facilities for high-value crops in Taiwan. As they are located in the interior space, their ventilation is not as effective as outdoor, resulting in higher moisture accumulation, while the soil, plant, and water in a greenhouse also provide a good environment for the airborne microorganisms to grow. As a result, bioaerosols are easy to propagate and accumulate in the greenhouse. These microorganisms can be spread through such channels as workers, air ventilation, and irrigation water. Among them, workers in greenhouses are particularly vulnerable because they are directly exposed to bioaerosols during their work process. Although there is no report of hazardous microbial infection case from greenhouse contacts, considerable attention has been paid to respiratory symptoms caused by high microbial exposure in recent years.

Several studies had explored the relationship between the distribution of bioaerosol concentration in greenhouses and health effects. Radon et al. (2002) conducted a study on 37 greenhouses in Spain and found that the fungi concentration was  $8.3 \times 10^4$  CFU/m<sup>3</sup>, while bacteria concentration was  $4.1 \times 10^4$  CFU/m<sup>3</sup>[1]. Mons (2004) found that in the sampled greenhouses, the fungi concentration was 1,700-11,000 CFU/m<sup>3</sup>, bacteria concentration was 770-5,200 CFU/m<sup>3</sup>, and toxin concentration within bacteria was 0.17- 0.89 EU/m<sup>3</sup>[2]. Hansen et al (2010) measured bioaerosols in the greenhouses for planting tomatoes, in which total bacteria concentration in the air was  $9.6 \times 10^2$ -  $3.4 \times 10^4$  CFU/m<sup>3</sup> and the total fungi concentration was  $2.6 \times 10^4$  -

$3.7 \times 10^5$  CFU/m<sup>3</sup>[3]. Adhikari et al. (2011) found that in the flower greenhouses, the bacteria and fungi concentration was between  $10^2$ - $10^5$  CFU/m<sup>3</sup>, and the toxin within bacteria was between  $10^1$  and  $10^3$  EU/m<sup>3</sup>[4].

Research papers on the health of 4,108 workers hired by greenhouses for planting flowers and food crops showed that they may suffer from such symptoms as skin itching, asthma, allergic bronchitis and dermatitis[5]. Researches also pointed out that 30% of greenhouse workers suffered from symptoms of respiratory allergies due to such fungi as Cladosporium, Penicillium, Aspergillus and Alternaria. Among them, 20% were found to have suffered from occupational asthma[2,6]. The US National Institute for Occupational Safety and Health (NIOSH) conducted a census on occupational health in 1995 and found that greenhouse workers had the highest ratio of the upper respiratory tract and the lower respiratory tract symptoms among all the hired farm workers[7].

These papers showed that greenhouses had quite high bioaerosol concentration and greenhouse workers suffered from health hazards. As similar studies are relatively scarce in Taiwan, this study aims to investigate the bioaerosol distribution in greenhouses. According to the pervious investigation, this study would not only investigate how the bacteria and fungi bioaerosol concentrations are distributed in the greenhouses but also identify what major strains are active in the greenhouses. Moreover, the environmental characteristics of the greenhouse facilities would also be conducted for evaluating their effects on

the bioaerosol distributions..

## Methodology

### 1. Sampling plans for greenhouses

Greenhouses for high-value crops in Taiwan are commonly of three types, namely the Holland Venlo type greenhouses, the steel reinforced Ya-tubed greenhouses and the Ya-tube-reinforced plastic sheeting greenhouses. All of them have a basic architecture with a closed environment, inside which the main factors affecting the bioaerosol distribution are still temperature, humidity, wind speed, environmental control facilities and outside air source. For sake of representation, three greenhouses in the Holland Venlo type and the Ya-tube-reinforced plastic sheeting structure were selected for this study and all of them are for planting high-value crops, including orchids and *Anoectochilus*. The sampling time was in June 2011.

Sampling methods inside greenhouses were decided with reference to specifications of the EPA NIEA E301.11C and NIEA E401.11C. The sampling height was 80-100 cm above the ground to simulate the breathing zone of greenhouse workers (considering that they normally bend down or sit on chairs when carrying out plant cultivation and conservation). The locations and the number of sampling sites were mainly decided based on the size of working area, work content, and pollution sources in the operating environment. Moreover, such indoor micro-environmental conditions at each sampling site as temperature, humidity, and wind speed were recorded to facilitate the judgement of

indoor air pollution sources and the assessments of human exposure.

Figures 1 to 3 are the plan views of greenhouses selected for this study. All the three greenhouses are indoor workplaces, on each of which a triplicate sampling was conducted in the morning, at noon and in the afternoon so as to compare the bioaerosol distributions at different time periods. Each greenhouse is equipped with such devices as cooling curtains and exhaust fans.

Exhaust fan					
Sampling site I		Sampling site J		Sampling site E	
		Walkway		Sampling site D	
Sampling site H					
				Sampling site C	
Sampling site G					
				Sampling site B	
Sampling site F		Sampling site K			
Water curtain		Entrance	Water curtain		
Sampling site A					

Figure 1 Plan view of Greenhouse 1

Exhaust fan	Sampling site H		Sampling site J		Sampling site D	Water curtain
			Walkway		Sampling site C	
	Sampling site G					
					Sampling site B	
	Sampling site F					
				Sampling site A		
	Sampling site E		Sampling site I		Sampling site K	
			Entrance			

Figure 2 Plan view of Greenhouse 2

Covered water curtain			
Sampling site E			Sampling site D
			Sampling site C
Office			Sampling site B
			Sampling site A
		Exhaust fan	Entrance
Sampling site F			

Figure 3 Plan view of greenhouse 3

## 2. Sampling of active bioaerosols

The Biostage Simple-stage Vcable Cascade Impactor (SKC Inc., USA) was used for sampling in this study. This Impactor has 400 holes with a pore size of 0.25 mm, which can draw air by starting the built-in motor powered by rechargeable batteries. When the air flow direction is changed, bacteria and fungi are collected onto a medium at a flow rate of 28.3 L/min. The Impactor itself can adjust its own air flow before and after the sampling. The sampling medium is disposable plastic petri dishes (90mm diameter) on which 27 mL of agar was poured. In this study, two kinds of agars were used, namely Malt Extract Agar (MEA) and Trypticase Soy Agar (TSA). The MEA is an agar recommended by American Conference of Government Industrial Hygienists (ACGIH) for most of fungi growth[8-9], while TSA is used to collect bacteria from the air for culture. Two samplings were conducted and repeated at each sampling site, one in the early morning during the slaughtering time and the other in the morning after the slaughter (non-slaughtering time). The samples were put into the incubator for culture. After pre-test, the sampling time was then set at 30 seconds to avoid collecting incalculable amounts of bacteria and fung.

The TSA and MEA are prepared by taking either medium at its standard amount in accordance with the formula, mixing them uniformly with an appropriate amount of deionized water, putting them into the 121°C pressurized autoclave to sterilize for 20 minutes, cooling the sterilized media to 55-60°C, dispensing 27 ml of either medium to the culture

dishes (90×15 mm), wait until the media solidify and then store them into a 4°C refrigerator for future use.

Before the number of colonies is counted, the TSA bacteria medium must be cultured at 30±1°C for 48±2 hours while the MEA fungi must be cultured inside an incubator of 25±1° C for 4±1 days. The number of colonies must be adjusted in accordance with the positive hole conversion table. Then, put such parameters as the adjusted number of colonies, sampling flow and sampling time into the formula below to estimate the air concentration and then the bioaerosol concentration in the operating environment of slaughtering greenhouses. According to the EPA standard methods, the minimum detection limit is less than 1 CFU as a basis for estimation. Sampling time of this study is 10 seconds, so the lower limit is <212 CFU/m<sup>3</sup>.

$$\text{Concentration (CFU/m}^3\text{)} = \text{Number of colonies (CFU)} / [28.3 \text{ (L/min)} \times t(\text{min}) \times 10^{-3}(\text{m}^3/\text{L})]$$

..... (1)

## 3. Strain identification

This work followed the pervious researches [10-11] to sample and purify the fungi and bacteria bioaerosols and then identify their strains by ways of molecular biology. Therefore, in this study we first sampled and cultured the bacteria and fungi. For the bacteria, we separated and purified those bacteria in the same patterns and then classified them and then used the Gram staining method to preliminarily identify their strains by molecular biology. For fungi, we also firstly separated and purified those fungi in the same patterns and then classified them and then purified them by

sub-colonization and identified their strains by molecular biology. For strains identification, bacteria were extracted of DNA for 16S rDNA comparison, while fungi were extracted of DNA for 18S rDNA comparison.

#### 4. Detection of environmental characteristics

During the sampling process, we also monitored the characteristics of the operating environment by using the anemometer (PROVA AVM-03 / AVM-01) to measure the wind speeds in poultry-slaughtering environment, and the Q-trak (Model 7565, TSI Inc., USA) to measure the temperature and relative humidity in the operating environment.

#### 5. Statistical test

In general, the distribution of bioaerosols is skewed to the right. Therefore, nonparametric statistics is applied in that the Kruskal-Wallis Test is used to conduct the statistical significance analysis for bioaerosol concentration in different operating areas at different work hours, the Wilcoxon Signed-Rank Test is used to conduct the statistical significance analysis for difference between indoor and outdoor bioaerosols, and the Spearman correlation coefficient is used to analyze how environmental conditions would impact the bioaerosol distribution.

### Results and discussions

#### 1. Distribution of bioaerosols in Greenhouse 1

In this study, Greenhouse 1 is for planting *Anoectochilus*. Its size is about 4 meters high, 22

meters long, and 10 meters wide. The average ventilation rate for the operating environment in Greenhouse 1 is 0.9 l/hr. which is estimated by the amounts of ventilation generated by the fan whose wind speed is about 4.5 m/s when it is in operation. However, the fan is not turned on at a fixed time but switched on/off by a temperature sensing device. There were 11 sampling sites in Greenhouse 1. Sampling site A was located outdoor while the other 10 sampling sites were set indoor.

Figures 4 and 5 are statistics showing the distribution of bacteria and fungi bioaerosol concentrations of the 11 sampling sites. Table 1 shows the environmental conditions during sampling. The results show the bacteria bioaerosol concentrations at the outdoor sampling site lie between  $254\pm 43$  and  $495\pm 104$  CFU/m<sup>3</sup>, while the bacteria bioaerosol concentrations at the indoor sampling sites lie between  $212\pm 33$  and  $1,413\pm 324$  CFU/m<sup>3</sup>, with the highest concentration appearing at the sampling site D at noon and the lowest concentration occurring at the sampling site C in the afternoon. For fungi bioaerosols, the results show the bacteria bioaerosol concentrations at the outdoor sampling site lie between  $495\pm 201$  and  $777\pm 211$  CFU/m<sup>3</sup>, while the fungi bioaerosol concentrations at the indoor sampling sites lie between  $565\pm 143$  and  $3463\pm 365$  CFU/m<sup>3</sup>, with the highest concentration appearing at sampling site I in the morning and the lowest concentration occurring at sampling site E in the afternoon.

Overall, the Wilcoxon Signed Rank test results showed that indoor bacteria bioaerosol concentrations was higher than the outdoor bacteria bioaerosol concentrations ( $p < 0.05$ ,

n=99). The Kruskal-Wallis Test used to analyze the indoor bacteria bioaerosol concentrations in different periods of time showed a result that the bacteria bioaerosol concentrations in the morning was higher than the other two periods ( $p < 0.05$ , n=99). When the Kruskal-Wallis Test was used to analyze the bacteria bioaerosol concentrations at different indoor sampling sites, the results showed no statistical significance ( $p > 0.05$ , n=99). If the bacteria bioaerosol concentrations at the bedstead planting area was compared

with that at the exhaust port area, the amount of bacteria bioaerosols also showed no statistical significance. The same results were shown in both the characteristics of overall fungi bioaerosols and bacteria bioaerosols. When the Spearman correlation coefficient was used to analyze the relevance between the environmental conditions (Table 2) and bacteria and fungi bioaerosol concentrations, the result showed no statistical significance, too.

Table 1 Environmental conditions at sampling

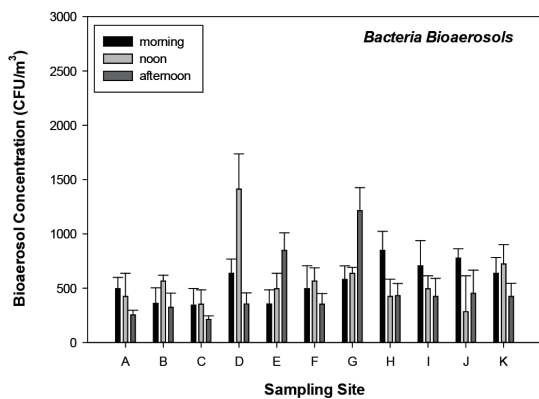


Figure 4 Distribution of bacteria bioaerosol concentrations in Greenhouse 1

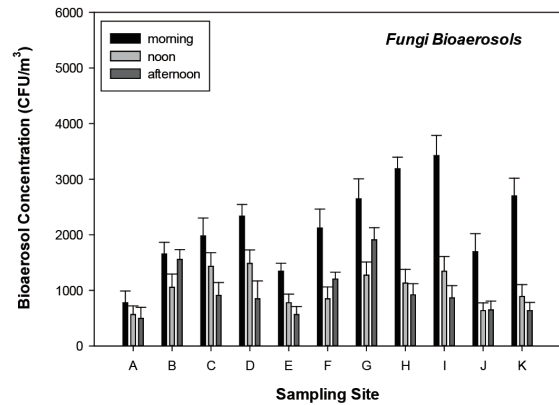


Figure 5 Distribution of fungi bioaerosol concentrations in Greenhouse 1

time in Greenhouse 1

Sampling site	Temperature (°C)			Humidity(%)			Wind speed(cm/s)		
	Morning	Noon	Afternoon	Morning	Noon	Afternoon	Morning	Noon	Afternoon
A (Outdoor)	29.1	33.0	29.2	80	67	80	0.11	0.15	0.13
B	29.1	33.1	29.0	81	68	80	0.15	0.18	0.18
C	29.0	33.0	29.1	80	67	81	0.12	0.16	0.15
D	29.1	33.2	29.0	80	67	80	0.14	0.19	0.23
E	29.2	33.0	28.9	79	68	81	0.14	0.17	0.15
F	29.0	33.1	29.0	80	69	82	0.09	0.19	0.18
G	29.0	33.0	29.0	81	68	80	0.13	0.20	0.14
H	29.1	33.1	29.1	78	67	80	0.14	0.18	0.11
I	29.0	33.0	29.2	79	67	80	0.11	0.19	0.18
J	29.0	33.1	29.0	80	68	81	0.15	0.21	0.18
K	30.1	33.5	29.5	77	66	79	0.08	0.09	0.15

\*n=99(11 sampling sites, where 3 environmental factors were measured in the morning, at noon, and in the afternoon, respectively)

In terms of strain identification, the results showed that fungi were mainly *Penicillium* spp., *Aspergillus* spp., *Acremonium* spp., and *Meira*

spp. The bacteria were mainly *Nocardiosis* spp., *Agromyces* spp., *Bacillus* spp., *Micrococcus* spp., and *Staphylococcus* spp..



## 2. Distribution of bioaerosols in Greenhouse 2

Greenhouse 2 is for planting orchids. Its size is about 4.5 meters high, 30 meters long, and 12 meters wide. The average ventilation rate for the operating environment in Greenhouse 2 is 1.2 l/hr. which is estimated by the amounts of ventilation generated by the exhaust fan whose wind speed is about 6 m/s when it is in operation. However, the fan is not turned on at a fixed time but switched on/off by a temperature sensing device. Wind in the working environment moves from the water curtain to the exhaust fan. 11 sampling sites were planned in Greenhouse 2. Sampling site K was located outdoor while the other 10 sampling sites were set indoor.

Figures 6 and 7 are statistics showing the distribution of bacteria and fungi bioaerosol concentrations of the 11 sampling sites. Table 2 shows the environmental conditions during sampling. The results show the bacteria bioaerosol concentrations at the outdoor sampling site lie between  $431 \pm 105$  and  $843 \pm 113$  CFU/m<sup>3</sup>, while the bacteria bioaerosol concentrations at the indoor sampling sites lie between  $212 \pm 54$  and  $1,674 \pm 296$  CFU/m<sup>3</sup>, with the highest concentration appearing at the sampling site J in the afternoon and the lowest concentration occurring at the sampling site C in the afternoon. For fungi bioaerosols, the results show the bacteria bioaerosol concentrations at the outdoor sampling site lie between  $1,145 \pm 211$  and  $3,932 \pm 536$  CFU/m<sup>3</sup>, while the fungi bioaerosol concentrations at the indoor sampling sites lie between  $1,214 \pm 325$  and  $6,432 \pm 981$  CFU/m<sup>3</sup>, with the highest concentration appearing at sampling

site F at noon and the lowest concentration occurring at sampling site A in the afternoon.

The Wilcoxon Signed Rank test results showed that indoor bacteria bioaerosol concentrations was higher than the outdoor bacteria bioaerosol concentrations ( $p < 0.05$ ,  $n = 99$ ). The Kruskal-Wallis Test used to analyze the indoor bacteria bioaerosol concentrations in different periods of time showed a result that there was no statistical significance among different periods of operating time ( $p > 0.05$ ,  $n = 99$ ). When the Kruskal-Wallis Test was used to analyze the bacteria bioaerosol concentrations at different indoor sampling sites, the results showed no statistical significance ( $p > 0.05$ ,  $n = 99$ ). If the bacteria bioaerosol concentrations at the bedstead planting area was compared with that at the exhaust port area, the amount of bacteria bioaerosols also showed no statistical significance.

The Wilcoxon Signed Rank test results showed that indoor fungi bioaerosol concentration was higher than the outdoor fungi bioaerosol concentration ( $p > 0.05$ ,  $n = 99$ ). The Kruskal-Wallis Test used to analyze the indoor fungi bioaerosol concentrations in different periods of time showed a result that the fungi bioaerosol concentration was higher than that at the other two periods of operating time ( $p > 0.05$ ,  $n = 99$ ). When the Kruskal-Wallis Test was used to analyze the fungi bioaerosol concentrations at different indoor sampling sites, the results showed no statistical significance ( $p > 0.05$ ,  $n = 99$ ).

When the Spearman correlation coefficient was used to analyze the relevance between the environmental conditions (Table 2) and bacteria

and fungi bioaerosol concentrations, the result showed no statistical significance.

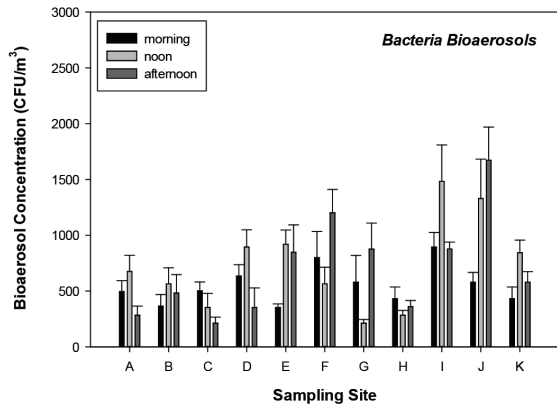


Figure 6 Distribution of bacteria bioaerosol concentrations in Greenhouse 2

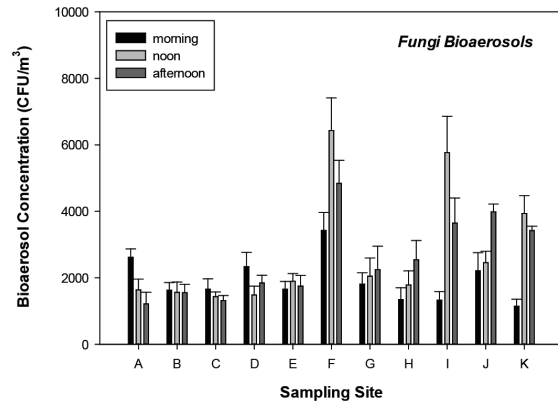


Figure 7 Distribution of fungi bioaerosol concentrations in Greenhouse 2

Table 2 Greenhouse 2 Environmental conditions at sampling time

sampling site	Temperature(°C)			Humidity(%)			Wind speed(cm/s)		
	Morning	Noon	Afternoon	Morning	Noon	Afternoon	Morning	Noon	Afternoon
A	30.0	32.1	29.3	81	83	87	0.13	0.15	0.22
B	30.0	32.1	29.4	81	84	86	0.15	0.16	0.14
C	30.1	32.2	29.3	82	84	86	0.16	0.16	0.24
D	30.2	32.3	29.5	81	83	87	0.15	0.18	0.21
E	30.1	32.1	29.4	82	83	87	0.12	0.21	0.13
F	30.1	32.1	29.4	82	83	88	0.11	0.11	0.15
G	29.9	32.2	29.5	81	85	87	0.13	0.14	0.16
H	30.0	32.0	29.4	81	83	87	0.13	0.15	0.22
I	30.1	32.1	29.4	81	83	88	0.14	0.16	0.16
J	30.2	32.1	29.3	80	83	88	0.15	0.19	0.14
K (Outdoor)	31.1	33.3	30.1	75	76	85	0.18	0.17	0.19

\*n=99(11 sampling sites, where 3 environmental factors were measured in the morning, at noon and in the afternoon, respectively)

The results showed that fungi were mainly *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., and *Acremonium* spp., and the bacteria mainly contained *Bacillus* spp., *Planobacterium* spp., *Chryseobacterium* spp., *Jeotgalicoccus* spp., *Staphylococcus* sp., and *Staphylococcus* spp..

### 3. Distribution of bioaerosols in Greenhouse 3

Greenhouse 3 is used for planting orchids. Its size is about 4 meters high, 15 meters long, and 10 meters wide. Greenhouse 3 is not ventilated but air-conditioned throughout the day. Its wind speed

in the real operating environment is 0.05-0.6 m/s. Six sampling sites were planned for Greenhouse 3. Sampling site F was located outdoor while the other sampling sites were set indoor.

Figures 8 and 9 are statistics showing the distribution of bacteria and fungi bioaerosol concentrations of the 6 sampling sites. Table 3 shows the environmental conditions during sampling. The results show the bacteria bioaerosol concentrations at the outdoor sampling site lie between 283±54 and 353±21 CFU/m<sup>3</sup>, while the bacteria bioaerosol concentrations at the indoor sampling sites lie

between  $283 \pm 54$  and  $1,272 \pm 209$  CFU/m<sup>3</sup>, with the highest concentration appearing at the sampling site E at noon and the lowest concentration occurring at the sampling site A at noon.

The Wilcoxon Signed Rank test results showed that there was no statistical significance between indoor and outdoor bacteria bioaerosol concentrations ( $p > 0.05$ ,  $n = 54$ ). The Kruskal-Wallis Test used to analyze the indoor bacteria bioaerosol concentrations in different periods of working time showed a result that there was no statistical significance among the bacteria bioaerosol concentrations in different periods ( $p > 0.05$ ,  $n = 54$ ); When the Kruskal-Wallis Test was used to analyze the bacteria bioaerosol concentrations at different indoor sampling sites, the results showed no statistical significance ( $p > 0.05$ ,  $n = 54$ )

In Greenhouse 3, the fungi bioaerosol concentrations at the outdoor sampling sites lie between  $1,032 \pm 231$ - $1,564 \pm 325$  CFU/m<sup>3</sup>, while the fungi bioaerosol concentrations at the indoor sampling sites lie between  $1,019 \pm 201$ - $2,987 \pm 312$  CFU/m<sup>3</sup>, with the highest concentration appearing at sampling site B in the afternoon and the lowest concentration occurring

at sampling site C in the morning.

The Wilcoxon Signed Rank test results showed that indoor fungi bioaerosol concentration was higher than the outdoor fungi bioaerosol concentration ( $p < 0.05$ ,  $n = 54$ ). The Kruskal-Wallis Test used to analyze indoor fungi bioaerosol concentrations at different periods of operating time showed a result that the number of fungi bioaerosols at noon was significantly higher than that at the other two periods ( $p < 0.05$ ,  $n = 54$ ). When the Kruskal-Wallis Test was used to analyze the fungi bioaerosol concentrations at different indoor sampling sites, the results showed no statistical significance ( $p > 0.05$ ,  $n = 54$ ).

When the Spearman correlation coefficient was used to analyze the relevance between environmental conditions (as shown in Table 3) and the bacteria and fungi bioaerosol concentrations, the results showed that there was moderate correlation (correlation coefficient  $r = 0.412$ ,  $p < 0.05$ ) between relative humidity and fungi bioaerosol concentrations, but the correlation analysis between the other environmental factors and the bacteria and fungi bioaerosols showed no statistical significance.

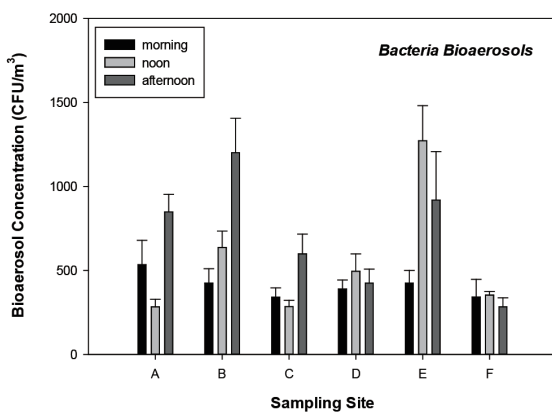


Figure 8 Distribution of bacteria bioaerosol concentrations in Greenhouse 3

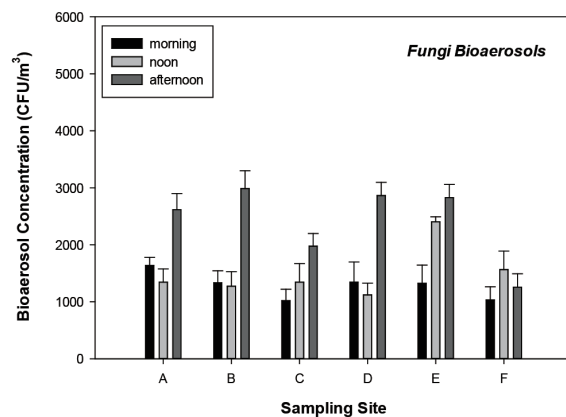


Figure 9 Distribution of fungi bioaerosol concentrations in Greenhouse 3

Table 3 Environmental conditions at sampling time in Greenhouse 3

sampling site	Temperature(°C)			Humidity(%)			Wind speed(cm/s)		
	Morning	Noon	Afternoon	Morning	Noon	Afternoon	Morning	Noon	Afternoon
A	29.1	28.0	22.1	60	62	77	0.03	0.05	0.05
B	29.1	28.1	22.2	61	62	78	0.04	0.04	0.06
C	29.2	28.2	22.3	60	61	77	0.03	0.03	0.05
D	29.3	28.1	22.2	62	61	77	0.03	0.04	0.06
E	29.1	28.0	22.1	60	61	77	0.03	0.05	0.06
F (Outdoor)	30.1	30.5	28.8	62	66	68	0.11	0.12	0.12

\*n=54(6 sampling sites, where 3 environmental factors were measured in the morning, at noon and in the afternoon, respectively)

The results of strain identification showed that the fungi bioaerosols were mainly *Penicillium* spp. and *Aspergillus* spp. while the bacteria bioaerosols were mainly *Bacillus* spp., *Planobacterium* spp., *Chryseobacterium* spp., *Jeotgalicoccus* spp., and *Staphylococcus* spp..

When the sampling results from the 3 greenhouses in Taiwan were compared with the results from foreign research papers[2-4], it was found that the bacteria and fungi bioaerosol concentrations in Taiwan's greenhouses were lower than those shown in research papers from foreign countries. Major species *Penicillium* spp. and *Aspergillus* spp. were found in both foreign research papers[2,6] and this study. The fungi bioaerosol concentration in greenhouses 2 and 3 where orchids are planted was significantly higher than that in Greenhouse 1, where *Anoectochilus* is planted. When the impacts of environmental factors were analyzed, the results from the 3 greenhouses showed that only the relative humidity appeared moderately correlated to the fungi bioaerosol concentrations, indicating that high relative humidity may affect the bioaerosol concentration.

In terms of strains, fungi include *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., *Acremonium* spp., and *Meira* spp. Among them, *Trichoderma* spp. has not been reported to impact human health

by any research papers. *Aspergillus* spp., which is genus of allergic *Aspergillus* disease, may cause opportunistic infections, with such symptoms as fever, cough, chest pain, and dyspnea. *Penicillium* spp. has no significant impact on human health. *Acremonium* spp. is an opportunistic pathogens. *Meira* spp. is a kind of mold that has not been reported by any literature to impact human health.

Bacteria include *Bacillus* spp., *Planobacterium* spp., *Chryseobacterium* spp., *Jeotgalicoccus* spp., *Staphylococcus* spp. *Nocardiopsis* spp., *Agromyces* spp., *Micrococcus* spp. Among them, *Bacillus* spp. is *Bacillus*, some of which can cause gastroenteritis, eye infections, and intravascular catheter-related sepsis. *Planobacterium* spp. is rod-shaped bacteria. *Chryseobacterium* spp. is *chryseobacterium*; *Jeotgalicoccus* spp. is *Natronococcus*; *Staphylococcus* spp. can often cause inflammation and septic to humans through wound infection. Some of *Nocardiopsis* spp. can cause lung infection or subcutaneous swelling pus. *Agromyces* spp. is a genus of *Actinomyces*, which has not been reported by any literature to affect human health. *Micrococcus* spp. is a *Micrococcus* genus, which has not been reported by any literature to affect human health.

#### 4. Conclusion

In the greenhouses, the highest average bacteria

bioaerosol concentrations was in the range of  $1,674 \pm 296$  CFU/m<sup>3</sup>, and the highest average fungi bioaerosols concentration was about  $6,432 \pm 981$  CFU/m<sup>3</sup>. The results showed that fungi bioaerosols were *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., *Acremonium* spp., and *Meira* spp., while bacteria bioaerosols were *Bacillus* spp., *Planobacterium* spp., *Chryseobacterium* spp., *Jeotgalicoccus* spp., *Staphylococcus* spp., *Nocardiosis* spp., *Agromyces* spp., and *Micrococcus* spp. On the whole, this study has found that domestic greenhouses have a lower bacteria and fungi bioaerosol concentration than the value reported by foreign research papers and strains of consistent characteristics. The results of sampling analysis showed that under the current ventilation conditions, each sampling site had high bioaerosol concentration, showing that ventilation must be increased to reduce bacteria and fungi bioaerosol concentrations to improve the working environment.

### Acknowledgements

Thanks to Institute of Occupational Safety and Health, Occupational Hygiene Division, Ministry of Labor, for financial assistance to this study (IOSH100-H314).

### References

- [1] Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham KJ, Palmgren U, Nowak D. Air contaminants in different European farming environments. *Annals of Agricultural and Environmental Medicine* 2002; 9: 41-8.
- [2] Mons E. Occupational asthma in greenhouse workers. *Current opinion in pulmonary medicine* 2004; 10: 147-50.
- [3] Hansen VM, Winding A, Madsen AM. Exposure to bioaerosols during the growth season of tomatoes in an organic greenhouse using Supresivit (*Trichoderma harzianum*) and Mycostop (*Streptomyces griseoviridis*). *Applied and environmental microbiology* 2010; 76: 5874-81.
- [4] Adhikari A, Gupta J, Wilkins JR, Olds RL, Indugula R, Cho KJ, Li C, Yermakov M. Airborne microorganisms, endotoxin, and (1→3)-β-D-glucan exposure in greenhouses and assessment of respiratory symptoms among workers. *Annals of occupational hygiene* 2011; 55: 272-85.
- [5] Illing HPA. Is working in greenhouses healthy? Evidence concerning the toxic risks that might affect greenhouse workers. *Occupational medicine* 1997;47: 281-93.
- [6] Monsó E, Magarolas R, Badorrey I, Radon K, Nowak D, Morera J. Occupational asthma in greenhouse flower and ornamental plant growers. *American journal of respiratory and critical care medicine* 2002; 165: 954-60.
- [7] Wilk V, Holden R. *New Directions in the Surveillance of Hired Farm Worker Health and Occupational Safety*. Cincinnati, OH: National Institute for Occupational Safety and Health; 1999.
- [8] Thorne PS, Kiekhaefer MS, Whitten P, Donham KJ. Comparison of bioaerosol sampling methods in barns housing swine. *Applied and environmental microbiology* 1992; 58: 2543-51.
- [9] Macher JM, Chatigny MA, Burge HA.

- Air sampling instruments for evaluation of atmospheric contaminants: Sampling airborne microorganisms and aeroallergens. ACGIH 1995, Cincinnati, Ohio. 589-617.
- [10] Acinas SG, Sarma-Rupavtarm R, Klepac-Ceraj V, Polz MF. PCR-induced sequence artifacts and bias: insights from comparison of two 16S rRNA clone libraries constructed from the same sample. *Applied and Environmental Microbiology* 2005; 71: 8966-9.
- [11] Chandler DP. Redefining relativity: quantitative PCR at low template concentrations for industrial and environmental microbiology. *Journal of Industrial Microbiology and Biotechnology* 1998; 21: 128-40.