2021 中華民國真菌學會年會暨真菌多樣性研討會

日期：2021 年 11 月 6 日 (星期六)

Webex 線上會議

會議名稱： **2021 中華民國真菌學會年會暨真菌多樣性研討會**

會議號碼：2514 490 4832

會議密碼：0000

時間 Time	大會議程 Program		主持人 Host
09:00 | 09:20	**線上報到 On-line registration**		
09:20 | 09:25	**開幕典禮 Opening Ceremony**		
09:25 | 10:00	鍾光仁 教授 國立中興大學	A dynamic and complex network connecting oxidative stress resistance, iron homeostasis and autophagy in *Alternaria alternata*	劉瑞芬 教授 國立臺灣大學 植物病理與微生物學系
10:00 | 10:35	彭家禮 教授 國立臺灣海洋大學	Mycobiota associated with Turtle Island Hydrothermal Vent Field, Taiwan	
10:35 | 10:45	**團體照拍攝及休息 Group photo & Tea break**		
10:45 | 11:20	黃尹則 助理教授 高雄醫學大學	諸神的美食：菌蠹蟲與菌蠹蟲真菌的共生關係 / Food of the gods: Ambrosia symbiosis between beetles and fungi	謝松源 主任 食品工業發展研究所 生物資源保存及研究中心
11:20 | 11:55	林國璽 博士 童綜合醫院	Surveillance of molecular epidemiology, antifungal sensitivity and pathogenicity of *Cryptococcus gattii* in Taiwan	
11:55 | 12:30	**午餐 Lunch**		

12:30 | 13:30	**線上理監事會議** **Online Board Meeting**		
13:30 | 14:00	**線上會員大會** **Online General Meeting of the Society Members**		
14:00 | 14:30	陳哲志 博士 中央研究院	Species diversity, taxonomy and multi-gene phylogeny of phlebioid clade (Phanerochaetaceae, Irpicaceae, Meruliaceae) of Polyporales	張碧芳 教授 國立中興大學 植物病理學系
14:30 | 15:00	張書林 助理教授 嘉南藥理大學	Engineered biosynthesis of natural products in *Aspergillus nidulans*	
15:00 | 15:10	**休息** **Tea break**		
15:10 | 17:00	會友研究成果發表 Member research presentation	學生口頭報告 Student oral presentation	沈偉強 教授 國立臺灣大學 植物病理與微生物學系
17:00 | **17:10**	**綜合討論及閉幕** **Discussion & Closing Remark**		

特邀專題演講一

Dynamic and complex network connecting oxidative stress resistance, iron homeostasis and autophagy in *Alternaria alternata*

Kuang-Ren Chung and Pei-Ching Wu
Department of Plant Pathology, College of Agriculture and Natural Resources, National Chung-Hsing University, Taichung, Taiwan

Necrotrophic fungi often deploy toxins or cell wall-degrading enzymes to kill host cells prior to invasion and thus are less affected by the hypersensitive reaction. Necrotrophic fungi may utilize ROS-induced damages to their advantage. The tangerine pathotype of *Alternaria alternata* relies on the production of a host-selective toxin to colonize its host plants. The ability to mitigate the toxicity of reactive oxygen species (ROS) also is required for pathogenesis. Considerable advances have been made in the understanding of the mechanisms by which *A. alternata* is protected from deleterious effects of ROS. *A. alternata* is capable of producing ROS by a membrane-bound NADPH oxidase (Nox), which plays regulatory roles during conidial formation and for ROS resistance and also has a substantial contribution to fungal virulence and to effective penetration of citrus hosts. A low level of ROS generated by Nox is critical for activating global regulators involved in the detoxification of ROS, cellular protection, and the biosynthesis of siderophores for iron acquisition. ROS detoxification is a complex process that involves multiple transcription regulators and signaling pathways. Studies have identified several proteins and pathways that are required for ROS resistance in the pathotype of *A. alternata*. Those include the Yap1 bzip transcription factor, the Skn7 response regulator, the Hog1 mitogen-activated protein kinase (MAPK)-mediated pathway, as well as peroxisome and autophagy-mediated processes. Through the coordinate balance of generating, sensing and detoxification of ROS, *A. alternata* can colonize within the citrus hosts.

特邀專題演講二

Mycobiota associated with Turtle Island Hydrothermal Vent Field, Taiwan

Ka-Lai Pang
Institute of Marine Biology and Centre of Excellence for the Oceans, National Taiwan Ocean University, Taiwan (ROC)

Fungi associated with marine hydrothermal vent ecosystems are little known. Diversity of fungi associated with sediment, seawater and animals collected at/near the marine shallow-water hydrothermal vents of Turtle Island, Taiwan was investigated. Fifty-four and Fifty-nine species of fungi were isolated from black and yellow (with sulfur) sediment, respectively. A total of 26 species were isolated from the vent crab *Xenograpsus testudinatus*. Ascomycota was the dominant taxa over Basidiomycota. Majority of the fungi recovered are terrestrial species but have been previously reported from the marine environment. A growth study of selected species under a range of pHs, salinities and temperatures revealed different responses of the fungi: (1) wide pH, salinity and temperature ranges, (2) salinity-dependent and temperature-sensitive, and (3) temperature-tolerant. A transcriptome analysis of the thermal-tolerant *Aspergillus terreus* NTOU4989 suggested its stress response to pH, salinity and temperature. Further examination of growth response of a number of terrestrial and marine isolates of *A. terreus* under different pHs (3, 7), temperatures (25 °C, 45 °C) and salinities (0 ‰, 30 ‰) categorized these isolates into three groups according to their ecological origin: marine hydrothermal vent, terrestrial and marine algal isolates. However, phylogeny of these isolates based on various protein genes including calmodulin, β-tubulin, elongation factor 1α and RNA polymerase II subunit did not correspond to their ecological origin. The marine environment may harbor both terrestrial and marine isolates of *A. terreus*, which have the physiological/genetic capacity to adapt to the marine conditions. Metabolic activity of *A. terreus* NTOU4989 was examined using BIOLOG FF MicroPlate™ under different pHs (3, 7), temperatures (25 °C, 45 °C) and salinities (0 ‰, 30 ‰) and in the presence of three metal ions in effluent of vents (Al^{3+}, Fe^{2+} and Mn^{2+}). Nitrogen-containing compounds were highly utilized over other types of carbon sources, such as monosaccharides. Monomers of lignin, cellulose and hemicellulose, and common sugars and amino acids of algae were utilized in selected conditions, providing evidence of a decomposer role of this fungus at the hydrothermal vent site.

特邀專題演講三

Food of the Gods: Ambrosia Symbiosis between Beetles and Fungi

Yin-Tse Huang 黃尹則
Assistant Professor, Department of Biomedical Science and Environment Biology, Kaohsiung Medical University.
ythuangmyco@kmu.edu.tw, ythuangmyco@gmail.com

Ambrosia symbiosis is a mutualistic lifeform between ambrosia beetles and ambrosia fungi. Ambrosia beetles are a group of specialized weevils excavate tunnels underneath tree barks, and cultivate their fungal associates, ambrosia fungi, as their sole food source. The ambrosia lifeform contains species, both beetles and fungi, that display a wide array of ecological forms in the environment. Some colonize the stressed or freshly dead trees and pose no significant impacts to the environment. Some prefer the fallen dead trees and facilitate wood decomposition. Some infest living trees and lead to enormous economic losses; examples such as avocado disease in the west USA, laurel wilt in the southeast USA, decline of tea trees in Sri Lanka, and oak wilt in Japan and Korea. These pestiferous species, though, account for only a small fraction of the overall ambrosia species (10 – 15 pestiferous beetle species out of 3200 known ambrosia species). Moreover, there are no apparent features for predicting their potential tree-killing capacity. Fundamental research such as the ecology, biology, and systematics of these diverse life forms are therefore essential to further our understanding of the symbiosis. As more is learned about their fundamental nature, we will be better able to prepare and prevent the next pestiferous species.

特邀專題演講四

Surveillance of molecular epidemiology, antifungal sensitivity and pathogenicity of *Cryptococcus gattii* in Taiwan

林國璽 博士
童綜合醫院

Cryptococcosis is a potentially fatal disease mainly caused by *Cryptococcus neoformans* species complex or *C. gattii* SC, which usually affects subjects with compromised immunity. After the outbreak of cryptococcosis in North America, attention to infections caused by *C. gattii* SC had risen. However, the rarity of human cryptococcosis caused by *C. gattii* SC limited the studies for this fungal pathogen. Studying the environmental strains is an alternative way to understand *C. gattii* SC. In this study, we developed a two-step method with a newly designed selective medium and used it to isolate *C. gattii* SC from the environment in Taiwan without the interference of coexisting filamentous fungi. Strains of *C. gattii* SC can be isolated in their tree habitats in Taiwan. Seven sequence types (STs) were identified, 4 in the VGI group and 3 in the VGII group. Three of the seven STs were newly identified in this study. According to the molecular epidemiology and geographical distributions, the clinical strains are closely related to the environmental strains. The phylogenetic analyses revealed that the *C. gattii* SC strains in Taiwan might have the same origin as those in South America and South Asia. The antifungal susceptibility tests demonstrated a significant difference in antifungal susceptibility between strains of different sequence types. The pathogenicity and virulence tests disclosed that all the environmental strains were pathogenic, and the difference of virulence between strains of each sequence type was significant. The newly identified ST 630 strains have the lowest antifungal susceptibility and the highest virulence among the strains of STs in the VGI group.

The newly developed two-step method provides an effective way to study the environmental strains of *C. gattii* SC, and the results of this study provided important information on molecular epidemiology, antifungal susceptibility and virulence of *C. gattii* SC in Taiwan.

特邀專題演講五

Species diversity, taxonomy and multi-gene phylogeny of phlebioid clade (Phanerochaetaceae, Irpicaceae, Meruliaceae) of Polyporales

Chen, Che-Chih[1, 2, 3], Chen, Chi-Yu[2], and Wu, Sheng-Hua[2,3]
[1]Biodiversity Research Center, Academia Sinica, Taipei, Taiwan
[2]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan
[3]Department of Biology, National Museum of Natural Science, Taichung, Taiwan

The phlebioid clade is a large group of the Polyporales (Basidiomycota), comprising of three families (Phanerochaetaceae, Irpicaceae, and Meruliaceae) and about 54 genera. Most species are corticioid fungi and resupinate polypores, the rest are pileate polypores and hydnoid fungi. Members of the phlebioid clade play an essential role in the maintenance of forest ecosystems. Most species in the phlebioid clade are saprotrophs on dead wood, causing a white rot. Nevertheless, some species may be endophytic, pathogenic on trees and symbiotic with other organisms, such as mycoheterotrophic orchids and ambrosia beetles. Despite the importance of the phlebioid clade, compared to the antrodia and core polyporoid clades of Polyporales, it has not been intensively studied. Generic delimitation within this clade is still not settled. Some genera with abundant species are known as paraphyletic or polyphyletic, and their members are scattered in different lineages, not fully consistent with the morphological features. Furthermore, there are still a considerable number of new species (especially corticoid species) requiring description, and molecular sequences are lacking for many known species. Thus, it is essential to provide a comprehensive investigation on a broad overview of the phlebioid clade, based on more studied taxa and multi-gene analyses. The present study used morphological and phylogenetic approaches to revise the generic classification of the phlebioid clade and survey species diversity. The phylogenetic analyses were performed using sequences of multiple genes, including the nuc rDNA ITS1-5.8S-ITS2 (ITS), the D1-D2 domains of 28S rDNA (28S), the RNA polymerase II largest subunit (*rpb1*), the RNA polymerase II second largest subunit (*rpb2*), and the translation elongation factor 1-α (*tef1*). We overall recognize 57 genera including six new ones, describe 32 species including 26 new species belonging to 15 genera, and present 18 new combinations belonging to 12 genera. Descriptions, illustrations and notes of new species and some new records are provided, as well as identification keys to genera of each family.

特邀專題演講六

Engineered biosynthesis of natural products in *Aspergillus nidulans*

Shu-Lin Chang (張書林)
嘉南藥理大學

Fungal natural products are an important source of drugs in the pharmaceutical industry. The sequencing projects have revealed that many fungal genomes contain large numbers of nature product biosynthetic clusters for which the products are still unknown. This indicates that most fungal biosynthetic gene clusters are either silent or expressed at very low levels under normal laboratory growth conditions. Here, we conducted *Aspergillus nidulans* as a model strain to demonstrate some approaches to unlock cryptic gene clusters, to elucidate their corresponding products and pathways.

學生口頭報告目錄

會友研究報告目錄

S-01

咖啡果小蠹上之鐮孢菌多樣性

薛曉萱[1]、施欣慧[2]、陳啟予[1]
[1]國立中興大學植物病理學系
[2]行政院農業委員會林業試驗所蓮華池研究中心

咖啡果小蠹為目前世界上最重要之咖啡害蟲，由母蟲鑽進果實內並產卵，成蟲與幼蟲取食果實而造成危害。早期之研究曾發現咖啡果小蠹蟲體上具有大量之鐮孢菌，並以 *Fusarium solani* 為主，且認為此真菌和咖啡果小蠹有共生之關係。然而近年來鐮孢菌之分類因分子親緣分析之快速發展而有重大之改變，早期認知的菌種有必要更精確的重新鑑定。本實驗即常態性的調查特定咖啡園，採集不同成熟度果實內之咖啡果小蠹，分析其所攜帶之鐮孢菌種類，研究結果發現鐮孢菌之種類極為多樣，包括 *Fusarium solani* species complex 有 5 種、*Fusarium fujikuroi* species complex 有 3 種、及 *Fusarium lateritium* species complex 有 3 種，不僅種類與文獻記載截然不同，且有地域上之差異，未來將持續針對咖啡果小蠹與鐮孢菌種類間是否具有共生關係進行探討。

S-02

番茄萎凋病菌鈣調磷酸酶之下游標靶 *CRZ1* 基因功能之探討

謝岳儒[1]、陳穎練[1]

[1]國立台灣大學植物病理與微生物學系

番茄是世界上廣泛種植的蔬菜之一。番茄萎凋病乃由尖鐮孢菌*Fusarium oxysporum* f. sp. *lycopersici*引起，是重要的植物真菌性病害之一，可造成嚴重的經濟損失。番茄萎凋病通常發生在潮濕和高溫的夏季，常見的病徵為葉片黃化、植株萎凋及維管束褐變。Crz1 是一種鋅指轉錄因子，在許多真菌物種中為鈣調磷酸酶(calcineurin)的重要下游標靶。在鈣調磷酸酶迅號路徑中，鈣調磷酸酶將Crz1去磷酸化，使Crz1得以進入細胞核來調控下游基因，例如與真菌發育，細胞壁完整性和離子平衡有關的基因表達。然而，Crz1在番茄萎凋病菌中的功能尚未被闡述。在本研究中，我們利用同源重組的技術剔除番茄萎凋病菌之*CRZ1*基因，並研究其功能。同時，本研究將透過各種壓力測試，包括滲透壓，金屬陽離子與細胞壁完整性，以瞭解Crz1在不同壓力條件下扮演之角色。另也將透過RNA定序與染色質免疫共沈澱結合芯片技術，尋找Crz1調控之基因，並藉由盆栽接種試驗，研究Crz1在番茄萎凋病菌中所扮演的毒力角色。透過胺基酸序列比對，找到了兩個可能為Crz1的同源基因*FOXG_00040*及*FOXG_05246*，並利用同源重組的技術獲得了兩者的突變株。前人研究顯示，Crz1會透過特定的motifs (PxIxIT, LxVP)與鈣調磷酸酶相互作用，在*FOXG_00040*上發現PxIxIT及LxVP，而*FOXG_05246*則無。此外，在鈣離子的刺激下，FOXG_00040聚集於細胞核而FOXG_05246則無，推測*FOXG_00040*極有可能為*CRZ1*基因。Δ*FOXG_05246*突變株於馬鈴薯葡萄糖瓊脂培養基(PDA)上較野生株生長緩慢、菌落扁平及產生較少的氣生菌絲，而Δ*FOXG_00040*突變株生長則與野生株相似。壓力測試的結果初步顯示兩突變株在滲透壓，金屬陽離子及細胞壁完整性壓力下與野生株相似，僅Δ*FOXG_05246*突變株於氧化壓力下較野生株生長稍微緩慢。盆栽試驗顯示Δ*FOXG_05246*突變株的毒力明顯下降，有趣的是，Δ*FOXG_00040*突變株的毒力反而比野生株還要強。總而言之，依據實驗結果初步判斷*FOXG_00040*為番茄萎凋病菌之*CRZ1*基因，*FOXG_00040*在番茄萎凋病致病能力上可能扮演著反向調控的角色。

關鍵字：番茄萎凋病菌，鈣調磷酸酶，Crz1，轉錄因子

S-03

龍船花葉片腺體上之黑殭菌

Metarhizium koreanum on glands of leaves of *Clerodendrum kaemferi*

薛曉萱、陳啟予
國立中興大學植物病理學系

龍船花(*Clerodendrum kaempferi*)為馬鞭草科之灌木，普遍存在於台灣之低海拔森林冠層之下，紅色的管狀花、及葉背密佈之盾狀腺體(peltate glands)為此植物之特色。本實驗發現，各地採集之龍船花植株之盾狀腺體上都可以發現黑殭菌—*Metarhizium*，植物葉片外觀完全健康，但顯微觀察後可發現有些盾狀腺體呈現褐化之現象，並可在褐化組織上出現 *Metarhizium* 真菌，顯然此真菌具有病原性，但只專一的感染盾狀腺體。經由形態觀察及 ITS、beta-tubulin、RPB1 之序列分析，將此真菌鑑定為 *Metarhizium koreanum*，而此菌僅曾在韓國、泰國、及日本被發現，並為飛蝨(planthopper)之病原菌，而此真菌在台灣卻與龍船花有密不可分之關聯性。

S-04

Molecular mechanisms of regulation of antifungal drug resistance by CZT-1

Yu-Tung, Lai[1], and A. Pedro Gonçalves[1,#]

[1]Department of Cell Biology and Anatomy, National Cheng Kung University, Tainan, Taiwan

[#]Email address: pedro@gs.ncku.edu.tw

It is estimated that there are approximately 1 billion cases and 1.5 million deaths from fungal infections worldwide, each year. Besides, fungal infections also affect animal and plants, with examples of fungal diseases causing massive death in staple grains, which leads to a heavy impact in regional economies and livelihoods. Despite the fact that some types of antifungal drugs are available for the treatment of fungal infections, their variety is insufficient. Therefore, understanding the mechanisms of antifungal drug resistance is an urgent issue. Previous research using *Neurospora crassa* as a model fungus demonstrated an association between *czt-1* and resistance to the cell death inducer staurosporine. The transcription factor gene *czt-1* regulates the expression of ATP-binding cassette transporters, including the gene encoding ABC-3. Expression levels of *czt-1* and *abc-3* are positively correlated with drug resistance, and the lack of *czt-1* or *abc-3* leads to hypersensitivity to staurosporine. In a wild population, a single nucleotide polymorphism in czt-1 was linked to the expression of another transcription factor, *tah-3*, which appears to also contribute to drug resistance. Moreover, a point mutation in *czt-1* (L680F) was found to explain the increased tolerance of the *acr-3* mutant strain to certain drugs. I propose to further explore the relationship between *czt-1* and *tah-3* and to analyze the functional consequences of the L680F mutation. Altogether, we plan to investigate the molecular mechanisms of *czt-1*, *tah-3* and their target genes and potentially recommend new therapeutic strategies to overcome antifungal drug resistance.

S-05

Exploring the epigenetic-associated fungal development through profiling DNA methylome and transcriptome of *Termitomyces*

Yu-Chun Huang[1,2], Huei-Mei Hsieh[2], Yu-Ming Ju[2], and Pao-Yang Chen[2]

[1] Bioinformatics Program, Taiwan International Graduate Program, Academia Sinica, National Taiwan University, Taipei, Taiwan.

[2] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan.

DNA methylation is an epigenetic modification often associated with gene regulation in response to the genetic and environmental factors. It has been found in many fungal species with context-specific methylation due to the diverse usage of DNA methyltransferases across fungal phyla. Mycelium was consistently found to display a lower methylation than other tissues in several fungal species, implying potential stage-specific DNA methylation in fungi. *Termitomyces eurrhizus* is an edible fungus having a symbiotic relationship with black winged subterranean termite. As the mushroom formation of *Termitomyces* is complicatedly triggered by external conditions, we hypothesized that the growth process of *Termitomyces* may be epigenetically influenced by genome-wide DNA methylation. We assembled the *Termitomyces* genome, then profiled genome-wide DNA methylation at single-nucleotide resolution throughout five developmental stages. Our results indicated that the DNA methylation is mostly found at transposons and depleted from genebodies. We observed clear changes in the global methylation level throughout the course of mushroom forming, in particular at non-CG cytosines. Primordium, as the initiation stage of fruiting, has the highest methylation level than those from other tissues, with the differentially methylated regions specifically enriched in transposons and intergenic regions, mostly at non-CG cytosines. Intriguingly, although the transposons are abundantly methylated, we found that the transposons around highly expressed genes are mostly hypo-methylated; suggesting a potential gene regulation by the nearby transposons. Along with the stage transition from non-fruit body to fruit body, we identified specific sets of genes including several known fruiting genes where the changes of gene expression are also anti-correlated with the changes of non-CG methylation at their nearby transposons. This suggested that the stage-specific non-CG methylation of transposons nearby the genes may be responsible for mushroom initiation as well as other growth processes.

Keywords: Epigenomics; DNA methylation; transposon; *Termitomyces*; Fungal development

S-06

Elucidating Dub module functions of SAGA complex in the human fungal pathogen *Candida glabrata*

Yue-Han Huang[1], Ying-Lien Chen[1]
[1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan

Candidiasis is one of the most important fungal diseases, and generally refers to diseases caused by *Candida* species in skin or mucosal organs. Biofilm formation in *Candida* species is critical for host infection, but the regulation of biofilm formation is limited. Besides, due to the innate antifungal drug resistance of *C. glabrata* and the difficulty in the development of antifungal agents, the frequent use of a few antifungal drugs had gradually increased the pathogenicity of non-*albicans Candida* species. Post-translational modification has an important function in characterizing heredity, and SAGA complex contains two different post-translational modifications including histone acetylation (HAT module) and deubiquitination (Dub module), which are decisive in gene regulation, and highly conserved in many organisms. Previous research in our laboratory found that the HAT module *ADA2* regulates *C. glabrata* oxidative stress tolerance, drug tolerance and cell wall integrity. Meanwhile, HAT module deletion mutants increased their virulence in a murine model of systemic infection, which is different from the results of other pathogenic fungi. The roles of Dub module (*UBP8*, *SGF73*, *SGF11* and *SUS1*) in *C. glabrata* SAGA complex is not yet understood, thus we further characterize its function in *C. glabrata*. We found that all Dub module mutants increased ubH2B level except *sgf73* mutant. Furthermore, mutants that increased ubH2B level were sensitive to cell wall perturbing agent SDS and antifungal drug amphotericin B, while exhibited decreased level of biofilm formation. Besides, *sgf73* mutant was susceptible to oxidative stresses, antifungal drugs and cell wall perturbing agents compared to wild type. By transcript analysis, the inhibition of *CTA1* was observed in *sgf73* mutant upon treating H_2O_2. In conclusions, the Dub module module of SAGA complex plays vital roles in regulating ubH2B level and biofilm formation, but the mechanism is not clear.

Keywords: *Candida glabrata*, SAGA complex, Dub module, *UBP8*

S-07

雞肉絲菇萃取物調節糖尿病視網膜病變潛力探討

張嫩心[1]、黃馨儀[1]、謝慧美[2]、朱宇敏[2]、李信昌[1*]
[1]臺北醫學大學 營養學院 保健營養學系
[2]中央研究院 植物暨微生物學研究所

糖尿病視網膜病變(Diabetic retinopathy, DR)是糖尿病晚期常見的併發症之一，DR也是造成糖尿病(Diabetes mellitus, DM)患者視力喪失的主要原因。研究發現長期高血糖會誘發發炎反應及自由基生成，導致DR及破壞人類視網膜上皮細胞 (ARPE-19) 調節視網膜結構與機能。雞肉絲菇 (*Termitomyces* mushrooms, TM)為與真菌栽培白蟻共生之獨特菇類，研究發現不同品系雞肉絲菇所含有生物活性成分，例如酚類、麥角固醇、多醣、神經源性腦苷脂、麥角甾烷等具有抗氧化、免疫調節、抗腫瘤、控制高脂血症和抗菌的潛在用途，然而目前尚無關於雞肉絲菇萃取物介入ARPE-19細胞對於DR之研究。本研究以冷凍乾燥雞肉絲菇樣品，加入液態氮研磨均質成為粉末後，加入95%乙醇得到雞肉絲菇酒精萃取物 (TM-EE)，爾後萃取物經減壓濃縮及凍乾後，再以等體積之乙酸乙酯與去離子水進行萃取，經分劃可得到乙酸乙酯萃取物 (TM-EA)及水相萃取物 (TM-EW)，三種萃取物並以UPLC分析麥角固醇、2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) 自由基清除能力與Folin-Ciocalteu檢測總多酚含量。結果顯示麥角固醇含量在TM-EE (4.56 ± 0.19 mg/g DW) 最高，TM-EA (1.83 ± 0.06 mg/g DW) 次之，TM-EW (4.7 ±0.07 ug/g DW) 最少。總多酚含量顯示TM-EW (8.37 ± 0.40 mg/g of gallic acid equivalence, GAE) 最高，TM-EE (3.79 ± 0.13 mg/g of GAE) 次之，TM-EA (2.23 ± 0.12 mg/g of GAE) 最少。DPPH自由基清除率以TM-EA (47.99 ± 0.07%) 最高，TM-EW (17.82 ± 2.45%) 次之，TM-EE (8.62 ± 1.51%)最少。MTT法分析細胞存活率顯示，ARPE-19細胞經TM-EE萃取物(6.25-400 ug/ml) 介入0~72小時後，並不會對細胞造成毒性。未來研究將以高葡萄糖誘發ARPE-19細胞產生阻抗，以進一步探討TM-EE萃取物是否具有調節糖尿病視網膜病變潛力及探討分子機轉。

關鍵字：糖尿病視網膜病變、雞肉絲菇、人類視網膜上皮細胞、ARPE19

S-08

Functional characterization of genes potentially involved in low virulence and slow growth of *Colletotrichum scovillei* T-DNA insertion mutant B42

Kuang-Heng Chen[1], Miin-Huey Lee[1]

[1] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan

The yield and quality of chili pepper, a kind of worldwide spice, can be influenced by many pathogens. *Colletotrichum scovillei* as a pathogen of pepper anthracnose is prevalent in Taiwan and causes severe yield loss of chili. To study genes that are involved in development and pathogenicity of this pathogen, T-DNA insertional mutagenesis library was created. B42 is one of the mutant strains and showed slower growth and lower virulence than wild-type strain. After inverse PCR analysis and DNA sequencing, T-DNA of B42 was found to insert the noncoding region between two genes, hyp866 and hyp582. Through bioinformatics analysis, hyp866 encodes a hypothetical protein and hyp582 encodes a protein carrying a functional domain that belongs to the superfamily of aminoglycoside phosphotransferase (APH). In the result of semi-Q RT-PCR, the two genes showed lower expression levels in B42 than wild-type strain when cultured on PDA. To understand the function roles of the two genes in the slow growth and low virulence of B42, gene knockout mutants of hyp866 and hyp582 were generated. Mutants of hyp866 appeared to grow slightly faster than wild-type strain on modified Cazpek's medium, while mutants of hyp582 were more resistant to hygromycin and geneticin (G418) than wild-type on MS agar medium. Compared to wild-type strain, the hyp866 knockout strains didn't show significant difference on virulence on chili pepper fruit.

S-09

蟻巢傘菌漆氧化酶在 *Pichia pastoris* KM71H 表現系統中異源表達與活性分析

翁仲毅[1]、賴吉永[1]
[1]國立彰化師範大學生物學系

漆氧化酶(EC 1.10.3.2)屬於多銅氧化酶的一員，以芳香族的酚類或胺類化合物為受質，產生的副產物只有水。在工業應用上已廣泛使用在造紙工業、汙水處理、脫色處理、紡織工業等，是一極具發展潛力的酵素。本實驗室利用養菌白蟻的共生真菌蟻巢傘菌(*Termitomyces*)作為多樣式漆氧化酶的來源，選殖台灣的 *Termitomyces* type Y 產生的每一種漆氧化酶異構型的核酸序列，利用異種宿主 *Pichia pastoris* 表現生產酵素。經實驗室團隊建構基因模型獲得漆氧化酶 cDNA 序列，再依據 *Pichia pastoris* 密碼子使用偏好，設計重組漆氧化酶基因 CYL5348，委託生技公司合成基因後連接 pPICZαA 表現載體。實驗結果顯示，使用 *Pichia pastoris* KM71H 菌株，添加 0.5%甲醇具有較佳活性，另添加 0.4mM 硫酸銅的第 7 天培養上清液，比起完全沒添加硫酸銅的活性提高約 26 倍，根據 SDS-PAGE 顯示蛋白質大小約 80kDa，以 ABTS 為受質，Native-PAGE 上觀察到呈色反應。利用 Protino Ni-IDA 1000 column 管住純化，回收率為 36%，比活性（U/mg）提高約 8.14 倍，以分光光度計測得的反應數據計算酵素動力學參數，結果為 K_m=0.059 mM 和 k_{cat}=2235.53 sec^{-1}。

S-10

建立台灣尾子菌類真菌之詮釋模式
Epitypification of cercosporoid fungi in Taiwan

范晏滋，陳啟予
國立中興大學植物病理學系

尾子菌類真菌指的是分類上屬於 Mycosphaerellaceae 科之不完全菌，此類真菌普遍造成植物之葉斑病，其中以 *Pseudocercospora* 屬之種類最為豐富。台灣紀錄之尾子菌類真菌多達 4 百多種，其中，以新種在台灣發表之種類多達約 150 種，但這些在台灣建立之新種，多數都是早期發表、無菌株保存、而無法有分子序列可供研究，成為現今研究上之障礙。「詮釋模式(epitype)」是解決方法，其定義為：一個物種，若其原始模式標本之材料有模糊、老舊問題或狀態不佳時，選擇另一份新的、並經過謹慎鑑定之「標本」來代表此物種。因為古老的標本已無法再分離獲得菌株，國際上普遍權宜的運用此規則，採集新鮮標本作為「詮釋模式」，然後，再從新鮮的標本純化獲得代表菌株。藉此，老舊學名、及無任何菌株保存及序列資料之學名，能因此建立代表菌株，而得以納入現代之分子親緣分析。本研究將針對台灣早期以新種發表之尾子菌類真菌進行採集，並將採集到之標本定義為詮釋模式，同時自此標本分離菌株、及進行解序，此菌株、及序列即可做為序列分析之代表，然後依形態、及序列分析重新檢視這些早期之新種。目前已獲得 7 種台灣早期發表之尾子菌類真菌新種，包括 *Pseudocercospora callicarpicola*、*Pseudocercospora clematidis*、*Pseudocercospora diospyricola*、*Pseudocercospora liquidambaris*、*Pseudocercospora pruni-yedoensis*、*Pseudocercospora viticis*、*Pseudocercosporella oxalidis*，除了同時分離獲得菌株、及解序外，持續將進行正式建立詮釋模式，期望能逐漸的完備台灣尾子菌類真菌之研究。

S-11

造成鳳梨釋迦果腐病之 *Botryosphaeria* spp. 多樣性調查及藥劑防治探討

李宗軒[1]、王誌偉[2]、Hiran A. Ariyawansa[1]、蔡恕仁[2]、藍天[1]、鍾嘉綾[1]
[1]國立臺灣大學植物醫學碩士學位學程
[2]行政院農業委員會臺東區農業改良場

鳳梨釋迦為我國重要外銷水果，由於果實為更年性，不耐長時間貯運，採收後發生真菌性病害亦為主要限制外銷的原因之一。根據2018及2019年採收後罹病果的菌相調查，*Botryosphaeria* 的分離率達36.7%及26.9%，其重要性僅次於 *Diaporthe* (分離率為40％及34.6％)。本研究於2020年挑選三處位於臺東市的鳳梨釋迦園，在不同生育時期及不同部位進行菌相調查，發現 *Diaporthe* 可於花期即潛伏感染於花器，*Botryosphaeria* 則可潛伏感染於成熟果的果梗與果實內部，並於果實後熟期間發病。挑選33株自田間及罹病果採集到的 *Botryosphaeria* 菌株，利用 ribosomal RNA internal transcribed spacer、translation elongation factor 1 alpha 及 beta-tubulin 序列建立多基因親緣關係樹，確認主要種類為 *B. fabicerciana* (87.9％)，次要種類為 *B. scharifii*、*B. ramosa* 及 *B. dothidea*，四種皆首次被報導為鳳梨釋迦病原菌，前三者為臺灣之新紀錄種。挑選四個種共14株代表性菌株接種於鳳梨釋迦果實，證實四種 *Botryosphaeria* spp. 不論有無傷口皆可感染果實，並產生與自然發病時相似的病徵。本研究挑選13種番荔枝核准登記使用的殺菌劑對四種 *Botryosphaeria* spp. 進行生體外 (*in vitro*) 藥劑篩選試驗，發現賽普護汰寧、百克敏 (含Salicylhydroxamic acid)、撲克拉錳、待克利、得克利及克熱淨 (烷苯磺酸鹽) 的抑制效果較佳，半效應濃度皆小於0.5 ppm，可應用於田間防治及採收後處理，減少果實腐損及檢疫問題。

S-12

Eremothecium 屬真菌、椿象及臺灣欒樹的相互關係

Eremothecium, Stinkbug and Taiwan Golden-rain Tree
— a Three-way Interrelation

李承軒，陳啟予
國立中興大學植物病理學系

Eremothecium 屬之真菌屬於酵母菌綱(*Saccharomycetes*)，是以菌絲型為主之酵母菌，部分種類在歷史上曾經造成棉花、柑橘及豆科等重要經濟作物的損失，為重要植物病原真菌。該屬真菌大多由半翅目的昆蟲所傳播，與昆蟲之間有緊密的共生關係，而臺灣並未有相關的文獻記載。本實驗室自臺灣欒樹的種子、無患子科植物相關之椿象體內，皆普遍可分離到不同種之 *Eremothecium* 屬真菌，此為臺灣發現 *Eremothecium* 屬真菌之首次記錄。並由高分離頻率、及高個體材料之帶菌率證明，*Eremothecium* 屬真菌、椿象、與臺灣欒樹三者，具有緊密之相互關係。

S-13

Evaluation of potentially anti-diabetic from edible mushroom (*Pholiota nameko)* extracts

Seng-Kai Vong [1], Yu-Ming Ju [3], Chiao-Ming Chen [2], Sing-Chung Li [1 *]

[1] School of Nutrition and Health Sciences, College of Nutrition, Taipei Medical University, Taipei, Taiwan

[2] Department of Food Science, Nutrition, and Nutraceutical Biotechnology, Shih Chien University, Taipei, Taiwan

[3] Institute of Plant and Microbial Biology, Academia Sinica, Taipei, 11529, Taiwan

Skeletal muscle plays an important role in glycaemic control and maintains glucose homeostasis. Impaired insulin action in muscles leads to insulin resistance (IR) and Type 2 diabetes (T2DM), as known as chronic metabolic disorder has been rapidly increasing all over the world. Reactive oxygen species (ROS) generated from oxidative stress is directly involved in IR in T2DM. *Pholiota nameko* (PN), a kind of edible and medicinal fungus. It is now widely cultivated in China and Japan for food and traditional medicine. Previously it has been reported that the phytosterol such as Vitamin D_2 (VD_2) and ergosterol (ERG) and antioxidant can improve IR in T2DM. But the hypoglycemic effect of PN is still unclear. Therefore, the objective of this study is to compare the phenolic and antioxidant activity from PN extract in different polar ratio, along with cell viability assessment through C2C12 myoblast cell. 5 grams of PN was ground into powder by liquid nitrogen and treated with 95% ethanol to obtain PN ethanol extract (PN-EE). Freeze-dried PN-EE sample was partition into ethyl acetate layer (PN-EA) and water layer (PN-EW). Subsequently, the VD_2 and ERG level of PN's extracts were examined by UPLC followed by MTT assay of cell viability. UPLC assessment shows that VD_2 level was high in PN-EE (12.23 ug/g DW) and low in PN-EA (4.53 ug/g DW), whereas, ERG level was high in PN-EA (0.84 mg/g DW) and low in PN-EE (0.66 mg/g DW). However, VD_2 and ERG cannot be detected in PN-EW through UPLC. Folin-Ciocalteu and 2,2-dipheny-1-picryl-hydrazyl-hydrate (DPPH) indicates both PN-EE and PN-EW have high phenolic contents (5 ± 0.17, 8.59 ± 0.3 mg of GAE /g) and antioxidant activity ($47.19 \pm 1.54\%$, $56.76 \pm 1.49\%$) compares with PN-EA. Furthermore, MTT assay was performed by applying PN-EE in concentration from 12.5 to 400 ug/mL for 24-48 hours. The result showed that PN-EE did not cause any toxicity in dose dependent manner. Finally, it is concluded that PN-EE/PN-EW have more therapeutic potential among all the extracts, with high phytosterol and antioxidant activity and it can be used to develop antidiabetic ingredients to improve ROS associated IR. In accordance with current study outcomes further work has been

pursuing to explore the molecular mechanism of PN-EE or PN-EW in IR muscle cell.

Keywords: *Pholiota nameko*, Ethanol extract, Water extract, phytosterol, Vitamin D_2, Ergosterol, C2C12 cell

R-14

A network-based method for predicting fungal essential genes through identification of core genes

Pei-Yu Lin[1], Yu-Chun Huang[1,2], Hsiao-Ching Lin[3], Wen-Neng Chou[4], and Pao-Yang Chen[1,2]

[1]Insititute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

[2] Bioinformatics, Taiwan International Graduate Program, Academia Sinica, National Taiwan University, Taipei, Taiwan

[3] Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

[4] Department of Botany, National Museum of Natural Science, Taichung, Taiwan

In fungal species, the essential genes are particularly helpful for the identification of antifungal drug targets, and the prediction of biosynthetic gene clusters. With resource expensive for experimentally constructing a catalog of essential genes, computational approaches to precisely identify candidate essential genes would be invaluable. Here, we present a network-based approach to predict fungal essential genes. To this end, a total of 491 fungal genomes (68% of assembled) kindly shared by communities were collected. We implemented Louvain algorithm for effectively clustering 6M fungal proteins from these fungal genomes based on sequence similarity, resulted in 67,826 orthologous gene clusters; each represents a group of similar proteins sharing amongst several fungal species. Each ortholog cluster was exploited as a sub-network where nodes are proteins and edges are the protein similarity. With their network statistics as parameters, a generalized linear model (GLM) and a random forest model were built to accurately rank these subnets by their likelihood of originating from an essential gene. We found that the top ranked subnets were of two types, one exhibits in many species (global) and the other is only vital for a few closed-relative fungi (local) with a higher network density, suggesting these local essential genes may be unique to specific yet close fungal families for living. For examples two of our predicted local essential genes, CFT1 which takes part in mRNA cleavage and UTP6 encoding for nucleolar proteins, are found to be close species well within genus *Nakaseomyces*. As a validation, our approach coupling with either GLM or random forest model reached 84% and 91% accuracy in predicting known essential genes from three well-studied species (*S. pombe*, *S. cerevisiae* and *A. fumigatus*). Additionally, GLM-based prediction tends find more previously undiscovered essential genes, for instance, several novel gene subnets associated with specific functions such as tetratricopeptide repeats coding protein or citrate synthase which have been shown to be essential for Eukaryotes. Our prediction strategy is based on a large number of fungal genomes, combining network biology, statistical modelling and machine learning, to provide a ranked list of fungal

core genes. The results as a web database would serve as a valuable resource for fungal genomic research.

Keywords: core genes, essential genes, fungi, orthologs

M-01

以人體肝臟 S9 酵素研究猴頭素 A (Erinacine A)體外代謝穩定性及其代謝物鑑定

郭育萱[1*], 李宗儒[1], 林定威[1], 陳勁初[1,2,3]

[1] 葡萄王生技股份有限公司

[2] 實踐大學食品營養與保健生技學系

[3] 中原大學生物科技系

猴頭菇(*Hericium erinaceus*)具有抗氧化、抗腫瘤、抗衰老、降血脂及保護胃黏膜等功效,先前研究發現其活性成分猴頭素 A(Erinacine A)在神經相關的疾病,如阿茲海默症、帕金森氏症及缺血性腦中風等皆有其顯著效果。因此,若要進入發展藥物的階段,代謝物鑑定就扮演著極重要的角色,不僅可作為改善藥物特性的參考,也可探討其安全性與功效。

本研究將猴頭素 A 與人體肝臟 S9 酵素在 37°C 下分別反應 0、10、30、60、120 分鐘後,再以乙腈終止反應並取出上清液待測。使用液相層析三段四極柱串聯式質譜儀(Liquid chromatography/triple quadrupole mass spectrometer, LC-QQQ/MS)進行定量分析,推算出猴頭素 A 代謝穩定性,再以超高效能液相層析四極柱飛行時間式質譜儀(Ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry, UPLC-QTOF/MS)分別在正、負模式下鑑定各時間點產生的代謝物。結果顯示,猴頭素 A 的半衰期為 126.37 分鐘,估計人體體外清除率(C_{Lint})為 87.02 mL/min/kg,肝清除率(C_{LH})為 32.48 mL/min/kg,肝萃取率(E_H)為 0.59;過程中產生的代謝物總計有 15 種。藉由了解猴頭素 A 的代謝過程及產生的代謝物,有助於未來促進新藥開發和精準醫學(precision medicine)的應用,及相關疾病的研究。

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M-02

液態發酵香杉芝菌絲體活性成分 antrodin C 在超臨界流體萃取及模擬移動床層析純化研究

朱心彤[1]、林定威[1]、梁明在[2]、陳勁初[1,.3, 4, 5]

[1]葡萄王生技股份有限公司

[2]義守大學 化學工程學系

[3]實踐大學 食品營養與保健生技學系

[4]國立台灣大學 食品科學系

[5]中原大學 生物科技系

香杉芝為台灣特有真菌，寄生於台灣特有的香杉樹(*Cunninghamia Konishii*)枯樹幹中，香杉芝與樟芝同屬多孔菌科(Polyporaceae)，薄孔菌屬(Antrodia)，因兩者外型極為相似，而樟芝價格昂貴，造成許多商人摻入型態相似但價格相較便宜的香杉芝，不過食用者日漸眾多，藥理活性經試驗後發現也不遑多讓。液態發酵的香杉芝菌絲體中發現許多的馬來酸衍生物，其中的潛力活性成分 Antrodin C，為具有抗癌幹細胞、護肝、可預防或治療糖尿病相關的心血管疾病、阿茲海默症等活性。本研究目的為開發超臨界流體(SF)萃取及模擬移動床(SMB)層析方法，量產純化香杉芝活性成分 Antrodin C。以 SF 萃取時，採用 95%乙醇當作輔溶劑，在萃取前 0.5 h 收集得到的萃取液，可做為高活性成分的商業產品；收集萃取第 0.5 h~2 h 收集得到的萃出物，則繼續做為下一階段層析純化的中間原料。SMB 的組態設計為 1-2/2/3，可以有效地將固定相再生，使用管柱尺寸為 5×30 cm，進料溶液濃度 50.00 g/L，在切換時間設定為 5.5 分鐘時，樣品回收率達 99%以上。研究結果顯示，在超臨界流體萃取後，經 SMB 純化其萃取物的最大處理量可達 550 公斤/年，相當適合邁向工業化量產製備，並能以更經濟環保的方式提供活性原料。