제24회

진균유전생물 컨퍼런스

2023. 2. 23 (목) ~ **24** (금)

덕산 스플라스 리솜

The 24th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2023

주최

한국미생물학회 진균유전생물학 분과

연세대학교 마이크로바이옴 연구원

한국미생물학회
The Microbiological Society of Korea

연세마이크로바이옴연구원
YONSEI MICROBIOME INITIATIVE

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YONSEI MICROBIOME INITIATIVE

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(사)한국미생물학회

서울시 강남구 테헤란로 7길 22, 한국과학기술회관 1관 810호

E-mail: msk@msk.or.kr

Tel.: 02-3453-3321

Fax: 02-3453-3322

Timetable

2.23 (Thu)	스테이타워 중연회장 SPACE B	
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13:10~13:20	Opening Ceremony	
13:20~14:00	Plenary Lecture	좌장: 반용선 교수 (연세대학교)
	채순기 교수 (배재대학교)	
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17:35~18:30	Dinner (스테이타워 더다이닝)	
18:30~19:55	S3. Fungal Biotechnology and Beyond	좌장: 최재혁 교수 (인천대학교)
	S3-1. 손문일 교수 (부산대학교) S3-2. 김신일 박사 (경상국립대학교) S3-3. 김 현 박사 (서울대학교) S3-4. Piyapat Rintarhat (중앙대학교)	
20:00~21:00	Group Photo & Poster Presentation & Mixer	

2.24 (Fri)	스테이타워 중연회장 SPACE B
09:00~12:00	General Meeting & Group Discussion
12:00	Closing Remark

Scientific Program

Plenary Lecture

PL Plenary Lecture

Chair: Yong-Sun Bahn (Yonsei University)

13:20-14:00

The Task of Hypoxia Adaptation in *Aspergillus*

Suhn-Kee Chae (Pai Chai University)

Symposium

S1 Human/Animal Pathogenic Fungi

Chair: Kyung-Tae Lee (Jeonbuk National University)

S1-1 14:00-14:30

SscA is a Key Transcription Factor for Conidiogenesis in *Aspergilli*

Hee-Soo Park (Kyungpook National University)

S1-2 14:30-14:45

A Study on the Reduction of Aflatoxin by Using *Aspergillus oryzae*

Yu-Kyung Kim (Woosuk University)

S1-3 14:45-15:00

Pleiotropic Roles of LAMMER Kinase, Lkh1 in Stress Responses and Virulence of *Cryptococcus neoformans*

Sunhak Kwon (Korea Atomic Energy Research Institute / Yonsei University)

S1-4 15:00-15:15

Comprehensive Insight into the Ras/cAMP/PKA Signaling Pathway in *Candida auris*

Ji-Seok Kim (Yonsei University)

S2 Plant Pathogenic Fungi

Chair: Hokyoung Son (Seoul National University)

S2-1 16:00-16:30

Distribution and Function of Members of the *AVR-Pita* Gene Family among Eight Clonal Lineages of *Magnaporthe oryzae* in the United States

Sook-Young Park (Sunchon National University)

S2-2 16:30-16:50

Functional Analysis of Essential Genes Using Heterokaryon

Yo-Han Ko (Jeonbuk National University)

S2-3 16:50-17:05

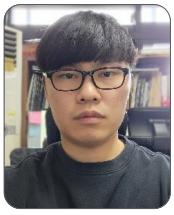
CRISPR-based Genetic Manipulation of *Aspergillus flavus* for Studying Drug Resistance

Sungyu Choi (Chonnam National University)

S2-4 17:05-17:20

A Transcriptomic and Physiological Analysis Revealed the Adaptive Mechanisms toward Oxidative Stress in the Plant Pathogenic Fungus *Fusarium graminearum*

Jiyeun Park (Seoul National University)



S2-5 17:20-17:35

Fungal Diversity Associated with Apple and Pear Seedlings and Canker Symtomatic Trees

HyeongJin Noh (Dankook University)

S3 Fungal Biotechnology and Beyond

Chair: Jaehyuk Choi (Incheon National University)

S3-1 18:30-19:00

Saccharomyces cerevisiae: A Sexy Yeast with a Prion Problem

Moonil Son (Pusan National University)

S3-2 19:00-19:20

Investigation of the Aromatic Compound Metabolic Pathway in *Cordyceps confragosa*

Sinil Kim (Gyeongsang National University)

S3-3 19:20-19:40

Mycobiome: Neglected Kingdom in the Microbiome Study

Hyun Kim (Seoul National University)

S3-4 19:40-19:55

Analysis of Human Gut Mycobiome: Culturomics and Sequencing Approaches

Piyapat Rintarhat (Chung-Ang University)

Plenary Lecture

PL

The Task of Hypoxia Adaptation in *Aspergillus*

Jun-Yong Kwak and Suhn-Kee Chae*

Department of Life Science, Pai Chai University

Hypoxia refers to low oxygen conditions and occurs in an ecological niche and host environment of *Aspergillus* fungi. Therefore, hypoxic adaptations of fungi are very important for their survival and pathogenicity. The sterol regulatory element-binding protein (SREBP), SrbA is an ER-tethered transcription factor required for hypoxia adaptation in *A. nidulans* and *A. fumigatus*. Although SrbA target genes have been identified in these species, how SrbA itself is regulated remains to be elucidated. We have screened numerous hypoxia-sensitive mutants induced by UV and identified a number of genes, including orthologs of SREBP (*srbA*), the Dsc complex (*dscA-E*), and rhomboid protease (*rbdB*) of *S. pombe.* Intriguingly, a gene encoding a putative signal peptide peptidase (*sppA*) was found to be a novel factor for hypoxia adaptation in *A. nidulans* and *A. fumigatus*. All played an essential role on the cleavage-activation of SrbA. SrbA is sequentially cleaved by RbdB likely associated with Dsc complex followed by SppA. These two proteases SppA and RbdB are localized at the different cell organelles of ER and Golgi, respectively. ER-to-Golgi transport of SREBP/Sre1 is controlled by SCAP/Scp1 in mammals and fission yeast. In contrast, *Aspergillus* species have no homologs of SCAP. Sec23 a component of COPII complex as a possible candidate responsible for the controlling SrbA transport from ER to Golgi in hypoxia will be discussed.

[Supported by the NRF grant funded by MSIT].

[S1] Human/Animal Pathogenic Fungi

S1-1

SscA is a Key Transcription Factor for Conidiogenesis in *Aspergilli*

Ye-Eun Son[1], Kyung Tae Lee[2], and Hee-Soo Park[1,3]*

[1]School of Food Science and Biotechnology, Kyungpook National University,
[2]Korea Zoonosis Research Institute, Jeonbuk National University,
[3]Department of Integrative Biology, Kyungpook National University

Conidiogenesis is the formation of asexual spores, called conidia, which are key propagates for filamentous fungi. The formation of conidia (or conidiophores) is controlled by various regulators and was mainly studied in the model fungus *Aspergillus nidulans*. Previous studies demonstrate that WetA, VosA, and VelB are key transcription factors for conidiogenesis. To further identify the spore-specific transcription factors, we performed the transcriptomic analysis in three *Aspergilli*. We found twenty-two spore-specific transcription factors and checked the roles of these transcription factors. Among them, we characterized one of the spore-specific-C_2H_2 zinc finger A SscA. Deletion of *sscA* led to defect conidia formation, maturation, viability, and germination. Further transcriptomic analysis found that SscA affects secondary metabolism and cell wall integrity. Moreover, the roles of SscA are conserved in two *Aspergillus* species including *A. fumigatus* and *A. flavus*. SscA affects conidia formation, viability, and pathogenesis in *A. fumigatus* and *A. flavus*. Taken together we proposed that SscA is a key spore-specific transcription factor in *Aspergillus* conidia.

S1-2

A Study on the Reduction of Aflatoxin by Using *Aspergillus oryzae*

Yu-Kyung Kim and Kap-Hoon Han*

Department of Pharmaceutical Engineering, Woosuk University

To study on the reduction of aflatoxin by using *Aspergillus oryzae* of Meju, Korean traditional soy brick, metagenomics analysis followed by fungal strain isolation were performed. Two different Meju samples, purchased in Gyeonggi province and Jeonbuk province, respectively, were subject to metagenome analysis and five different samples were used for fungal strain isolation. This study isolated and identified a total of 7 genus 30 filamentous fungi from the samples, which include *Aspergilli*, *Penicilli*, *Cladosporia* and *Mucor*, and confirmed the presence or absence of aflatoxin or ochratoxin production among the isolated fungi to avoid possible mycotoxigenic fungal isolates contamination. Moreover, ITS and 16S metagenomic sequencing were performed on some Meju samples to analyze the microbial distribution. Among the isolated strains, *A. flavus/oryzae* strains were examined for aflatoxin production by TLC method. The metagenome results showed that the distribution and abundance of fungi are very diverse according to location and production difference of the Meju samples. The ratio of commercially available yellow-koji molds and aflatoxin-producing strains was appropriately mixed to prepare Meju, and then aflatoxin production was analyzed by TLC method and HPLC method. Meanwhile, the isolated *A. oryzae* strain did not show the high amylase activity seen in the commercial yellow-koji strain, and the activity of lipase and peptidase did not show a significant difference between the strains. In addition, we found that the production of aflatoxin in Meju was reduced when *A. oryzae* and *A. flavus* strains were simultaneously inoculated and fermented.

S1-3

Pleiotropic Roles of LAMMER Kinase, Lkh1 in Stress Responses and Virulence of *Cryptococcus neoformans*

Sunhak Kwon[1,3], Ui Seung Kim[2], Kyung-Tae Lee[2], Yong-sun Bahn[3], and Kwang-Woo Jung[1*]

[1]Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute,

[2]Korea Zoonosis Research Institute, Jeonbuk National University,

[3]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Dual specificity LAMMER kinases are evolutionarily conserved in eukaryotes and play pivotal roles in diverse physiological processes such as differentiation. Although the roles of LAMMER kinase in fungal pathogens in pathogenicity and stress responses have been characterized, the function of LAMMER kinase in *Cryptococcus neoformans*, which is a human fungal pathogen and consider a model yeast of basidiomycetes, remains elusive. In this study, we identified a *LKH1* homolog, which encodes LAMMER kinase and constructed a strain deleted with *LKH1* and complemented strain. Similar to other fungi, the *lkh1Δ* mutant showed intrinsic growth defects. We found that *C. neoformans* Lkh1 was involved in diverse stress responses including oxidative stress and cell wall stress. Especially, Lkh1 regulated DNA damage response in Rad53-dependent and independent manners. Furthermore, the deletion of *LKH1* resulted in decreased basidiospores formation. We demonstrated that Lkh1 was hyper-phosphorylated under treatment of rapamycin, which is an inhibitor of TOR protein. Notably, the deletion of *LKH1* led to defects in melanin synthesis and capsule formation. Furthermore, we found that perturbation of *LKH1* attenuated the virulence of *C. neoformans* in the mouse model of cryptococcosis. Taken together, Lkh1 is required for stress responses, sexual differentiation and the virulence of *C. neoformans*.

[Supported by grants from NRF. No. 2020R1C1C1005468]

S1-4

Comprehensive Insight into the Ras/cAMP/PKA Signaling Pathway in *Candida auris*

Ji-Seok Kim[1], Kyung-Tae Lee[2], and Yong-Sun Bahn[1]*

[1]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University,
[2]Korea Zoonosis Research Institute, Jeonbuk National University

Candida auris is an invasive human fungal pathogen which cause high fatality disease in immuno-compromised patients. In addition, since *C. auris* has a multi-drug resistance, the importance of research on *C. auris* is increasing. Our recent study reported that adenylyl cyclase Cyr1 and protein kinase A (PKA) pathways play distinct and redundant roles in drug resistance and various pathobiological functions of *C. auris*, but its upstream and negative feedback regulatory mechanisms remain elusive. In this study, we focused on the upstream regulatory mechanisms of Ras/cAMP/PKA signaling pathway, which are believed to play an important role in the pathogenicity and drug resistance of pathogenic fungal species. Among the various genes related with the signaling pathway, we constructed knockout strains for the G-protein-coupled receptor Gpr1, G-protein alpha subunit Gpa2, RAS signal transduction GTPase Ras1, Guanyl-nucleotide exchange factor Cdc25, GTPase-activating protein Ira2, and cyclic nucleotide phosphodiesterase Pde1, Pde2. Then, we conducted a phenotypic analysis of each mutant to find out which genes are the main up-regulator of adenylyl cyclase Cyr1 in this pathway, and as a result, we found that Ras1 acts as the main upper regulator of Cyr1, not Gpr1 or Gpa2. The phenotypes of the Ras1 deletion strain and the Cyr1 deletion strain were generally similar, and the phenotypes were also very related to the Cdc25 deletion strain which regulates Ras1. We also confirmed that when the Ras/cAMP/PKA signaling pathway was inactivated, the drug resistance and growth rate was significantly reduced, and Sap activity involved in the pathogenicity of *Candida* species was remarkably decreased. Furthermore, we confirmed that the hyperactivation of Ras/cAMP/PKA signaling pathway can attenuate pathogenicity of *C. auris*. Consequently, these results will indicate that targeting Ras/cAMP/PKA signaling pathway could serve as an effective alternative to antifungal therapy against emerging multidrug-resistant fungal pathogen *C. auris*.

[S2] Plant Pathogenic Fungi

S2-1

Distribution and Function of Members of the *AVR-Pita* Gene Family among Eight Clonal Lineages of *Magnaporthe oryzae* in the United States

Sook-Young Park[1*], Chang Hyun Khang[2], James Correll[3], Yong-Hwan Lee[4], and Seogchan Kang[5*]

[1]Department of Agricultural Life Science, Sunchon National University,
[2]Department of Plant Biology, University of Georgia, Athens, GA 30602, USA
[3]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA
[4]Department of Agricultural Biotechnology, Seoul National University,
[5]Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA 16802, USA

Control for rice blast, caused by *Magnaporthe oryzae*, using resistance (*R*) genes is effective and environment friendly. However, frequent emergence of new races capable of evading deployed *R* genes due to changes in avirulence (*AVR*) genes breaks down this type of resistance. Here, we investigated distribution pattern, variation, and AVR function of members of the *AVR-Pita* gene family in eight clonal lineages of *M. oryzae* in the United States to understand the mechanism underpinning race variation caused by changes in this gene family. Probing 23 isolates that represent these lineages using three members of this gene family as probes revealed multiple unique genotypes, but limited variation was observed within each lineage. The copy number of *AVR-Pita1* ranged from 0 to 3. All isolates seem to lack *AVR-Pita2* but carry a single copy of *AVR-Pita3*. Novel members of the family may be present too. At least six distinct *AVR-Pita1* products are encoded. Three of them confer AVR function, making the strains expressing them avirulent to rice varieties containing *Pi-ta*. Comparison of the AVR-Pita1 sequences, their AVR function, and site-directed mutagenesis showed that tyrosine residue at the position 192 is essential for AVR function. Unlike previously characterized *AVR-Pita1*, which was located next to the telomere, most *AVR-Pita1* in the U.S. isolates may not be telomeric.

[This work was supported by a grant from the Rural Development Administration (PJ0152782023).]

S2-2

Functional Analysis of Essential Genes Using Heterokaryon

Yo-Han Ko, Jeesun Chun, Yeji Gwon, and Dae-Hyuk Kim*

Department of Molecular Biology, Department of Bioactive Material Sciences,
Institute for Molecular Biology and Genetics, Jeonbuk National University

Functional analysis of essential genes from chestnut blight fungus *C. parasitica* using null have shown that *C. parasitica* has a tendency of establishing heterokaryon, which contains two different types of nuclei in a common cytoplasm (i.e., one with the wild-type allele and the other with the null mutant allele). Although stable mycelial growth of the heterokaryotic transformant was observed on selective medium containing hygromycin B, neither germination nor growth of the resulting conidia, which were single-cell monokaryotic progeny, was observed on the medium. Through the germination rate of spores, it confirmed that the ratio of the two different nuclei of heterokaryon could be various by the selective pressure. *In trans* complementation of heterokaryons (heterokaryon rescuing) using a full-length wild-type as well as chimeric alleles resulted in complemented transformants that were able to grow on selective media for replacing and/or complementing markers. This study demonstrates that our fungal heterokaryon system can be applied effectively to determine whether a gene of interest is essential, to analyze terminal phenotype of a lethal gene, and to perform the functional analysis of a corresponding gene using complementation.

S2-3

CRISPR-based Genetic Manipulation of *Aspergillus flavus* for Studying Drug Resistance

Sungyu Choi, Doeun Son, and Hyunkyu Sang*

Department of Integrative Food, Bioscience and Biotechnology, Chonnam National University

Research on the control of fungi is vital to the global economy and health. *Aspergillus flavus*, a fungus that infects plants and animals, generates aflatoxin, which poses a hazard to the infected. Chemical treatments that inhibit the biosynthesis of ergosterol generated by the fungus (Azoles) or build pores by selectively binding to ergosterol (Amphotericin B) are frequently used to control *A. flavus*. Long-term accumulation of fungicide resistance diminishes effectiveness and generates over-dose or negative impacts. Previous research had established that azole resistance in *Aspergillus* spp. was caused by mutation(s) in the 14-sterol demethylase genes (*cyp51s*) or regulation of transcription factors (*atrR*, *srbA*, etc.). In the present study, CRISPR-CAS transformation technology was applied to study azole resistance in *A. flavus* by not only inhibiting the function of the genes aforementioned, but also directly inducing SNP(s) or indel(s) in the wild-type strain. In addition, we identified new SNPs/indels variants by comparing the genetic information of the *A. flavus* population and the fungicide phenotype in order to discover a new fungicide resistance mechanism. 'AlphaFold,' a new tool that predicts protein structure using machine learning, was also used to visualize drug target proteins and predict changes in antifungal drug binding affinity depending on base sequence variation. By combining newly developed molecular biology techniques with bioinformatics and machine learning tools, it is now possible to discover drug resistance mechanisms faster and more accurately than previous generations. This is anticipated to imply the potential for future drug development and effective fungus pathogen control.

S2-4

A Transcriptomic and Physiological Analysis Revealed the Adaptive Mechanisms toward Oxidative Stress in the Plant Pathogenic Fungus *Fusarium graminearum*

Jiyeun Park[1], Hyunhui Lee[2], Heeji Moon[1], Nahyun Lee[1], Soyoung Choi[1], Sieun Kim[1], Jung-Eun Kim[3], Yoonji Lee[1], Hun Kim[4], Gyung Ja Choi[4], Yin-Won Lee[1], Young-Su Seo[2], and Hokyoung Son[1,5]*

[1]Department of Agricultural Biotechnology, Seoul National University,
[2]Department of Integrated Biological Science, Pusan National University,
[3]Research Institute of Climate Change and Agriculture, National Institute of Horticultural and Herbal Science,
[4]Center for Eco-friendly New Materials, Korea Research Institute of Chemical Technology,
[5]Research Institute of Agriculture and Life Sciences, Seoul National University

Reactive oxygen species (ROS) are byproducts of normal cellular oxygen metabolisms and act as signaling molecules by modifying target proteins. However, excessive accumulation of ROS causes oxidative damage to various cellular biomolecules such as nucleic acids, proteins, and lipids. In plant cells, pattern-triggered-immunity- or effector-triggered-immunity-mediated ROS bursts prevent the plant pathogens from spreading to an adjacent cell. Meanwhile, pathogens have developed mechanisms to endure oxidative stress and successfully colonize plant cells. Therefore, it is important to unveil oxidative stress response (OSR) mechanisms of plant pathogens for developing efficient disease control strategies. In this study, we aimed to investigate the genetic and metabolic regulation of OSR in *F. graminearum* with two independent approaches. First, the transcriptome of six oxidative stress-sensitive mutants was analyzed to unravel the adaptation mechanism under oxidative stress conditions. Weighted correlation network analysis revealed that DNA damage response and ubiquitin-proteasome responses were up-regulated under oxidative stress conditions. Functional analysis of the "hub" genes characterized the important role of the heme biosynthetic mechanism in oxidative stress response of this fungus. Secondly, *FgbZIP007*, the most vulnerable genes to oxidative stress, were functionally characterized to explore the antioxidant mechanisms. Fgbzip007 is considered to affect the biosynthesis of glutathione, and we confirmed that glutathione metabolism is required for oxidative stress resistance and the pathogenicity of *F. graminearum*. Furthermore, ChIP-seq analysis identified a total of 118 genes as a target of Fgbzip007, and it revealed that Fgbzip007 is a global regulator of sulfur/cysteine and methionine metabolism. These studies provide comprehensive insights into various mechanisms by which fungi protect themselves and overcome oxidative stress.

S2-5

Fungal Diversity Associated with Apple and Pear Seedlings and Canker Symtomatic Trees

HyeongJin Noh[1], Ye eun Kim[2], Ye in Kim[1], Min Young Kang[1], Seong Jae Ahn[1], Hyun Uk Cho[1], and Seong Hwan Kim[1*]

[1]Department of Microbiology, College of Science and Technology, Dankook University,
[2]Plant Quarantine Technology Center, Animal and Plant Quarantine Agency

Plant canker is a common and widespread disease that causes sudden necrosis or long-lasting damage to trees or crops. Fungi are well known as the causal agents of diverse tree cankers. Severe cankers caused by fungi could lead to cut down fruit trees that have been grown for decades or to reduce fruit yields. Although some fungi such as *Diaporthe*, *Botryosphaeria*, *Nectria*, and *Cytospora* are known to cause cankers, systematic data on fungal diversity on cankered fruit trees are rarely available. In Kora, fruit tree cankers are continuously being reported accompanying with economic losses. Therefore, this study was performed to obtain information on the fungi inhabiting on seedlings and twigs of apple and pear trees which are cankered or have canker-like signs. As a result, a total of 573 isolates were obtained and classified as 36 genera and 44 species. Among the isolates, *Diaporthe eres* and *Botryosphaeria dothidea* accounted for a large proportion as canker-causing fungi. In addition, we found that *Neofusicoccum parvum*, *Paraconiothyrium brasiliense*, *Didymosphaeria rubi-ulmiformii*, and *Nothophoma quercina* are undescribed species associated with cankers of apple and pear trees in Korea. We also found that many seedlings are already infected with diverse canker-causing fungal species. We expect that our data could help to develop better management approaches for cankers caused by fungi on domestic apple and pear trees.

[S3] Fungal Biotechnology and Beyond

S3-1

Saccharomyces cerevisiae: A Sexy Yeast with a Prion Problem

Moonil Son*

Department of Microbiology, Pusan National University

Prions are infectious proteins, mostly having a self-propagating amyloid (filamentous protein polymer) structure consisting of an abnormal form of a normally soluble protein. These prions arise spontaneously in the cell without known reason, and their effects were generally considered as fatal based on prion diseases of human or mammals. However, the wide array of prion studies in yeast, *Saccharomyces cerevisiae*, including filamentous fungi revealed that their effects can range widely, from lethal to very mild (even cryptic) or functional, depending on the nature of the prion protein, and the specific prion variant (or strain) made by the same prion protein but with a different conformation. This prion biology is affected by an array of molecular chaperone systems, such as Hsp40, Hsp70, Hsp104, or their combination. In parallel with the systems required for prion propagation, yeast has multiple anti-prion systems, constantly working in the normal cell without overproduction or deficiency of any protein, which have negative effects on prions by blocking their formation, curing of many prions after arising, preventing prion infections and reducing the cytotoxicity produced by prions. From the protectors of nascent polypeptides (Ssb1/2p, Zuo1p and Ssz1p), to the protein sequester (Btn2p), the disaggregator (Hsp104), and the mysterious Cur1p normal levels of each can cure prion variants arising in its absence. The controllers of mRNA quality, nonsense-mediated mRNA decay proteins (Upf1, 2, 3), can cure newly formed prion variants by association with a prion forming protein. The regulator of inositol pyrophosphate metabolic pathway (Siw14p) cures certain prion variants by lowering the levels of certain organic compounds. Some of these proteins have other cellular functions (e.g., Btn2), while others produce an anti-prion effect through their primary role in the normal cell (e.g., ribosomal chaperones). Thus, these anti-prion actions are the innate defense strategy against prions. Here, we outline the anti-prion systems in yeast that produce innate immunity to prions by a multi-layered operation targeting each step of prion development.

S3-2

Investigation of the Aromatic Compound Metabolic Pathway in *Cordyceps confragosa*

Sinil Kim and Hyeon-Su Ro*

Department of BioMedical Bigdata (BK21) and Research Institute of Life Sciences, Gyeongsang National University

Phenylacetate (PA) is a major byproduct of the production of 6-aminopenicillanic acid, which is used as a precursor for the production of beta-lactam antibiotics, and the removal of PA in the pharmaceutical industry is one of the technical challenges. In this study, we attempted to isolate fungi of Ascomycota and Basidiomycota that can degrade PA effectively by using a minimal medium containing PA as the sole carbon source. As a result, we discovered a fungal strain FMGL1091, *Cordyceps confragosa*, which could effectively degrade PA. FMGL1091 was found to have both a PA-degradation pathway leading to 2,5-dihydroxy phenylacetic acid and 3,4-dihydroxy phenylacetic acid, and PA-degradation intermediate substrates were effectively degraded in subsequent minimal medium culture containing them as the sole carbon source. Transcriptome analysis of FMGL1091 cultured under PA conditions confirmed that cytochrome P450 monooxygenases and dioxygenases were expected to be involved in PA degradation, and verified the increased expression of these genes by RT-PCR. To clarify the functions of these genes, we first knocked out Orotidin-5'-phosphate decarboxylase (URA3) in FMGL1091. Next, we would like to confirm the function of each gene through PA-degradation products and intermediates analysis in cytochrome P450 monooxygenase and dioxygenase deletion strains produced using the uracil auxotrophic marker.

S3-3

Mycobiome: Neglected Kingdom in the Microbiome Study

Hyun Kim[1,2] and Yong-Hwan Lee[1,2,3,4,5]*

[1]Research Institute of Agriculture and Life Sciences, Seoul National University,
[2]Department of Agricultural Biotechnology, Seoul National University,
[3]Interdisciplinary Program in Agricultural Genomics, Seoul National University,
[4]Center for Plant Microbiome Research, Seoul National University,
[5]Plant Immunity Research Center, Seoul National University

The advancement of sequencing technology and omics tools allows researchers to delve into microbial communities in environments and organisms including humans, animals, and plants. Researchers have introduced the new term Microbiome, defined as microbial communities in a particular niche (microbiota) and the collective information on genomes, activity of microbes, and environmental conditions. Since the era of the microbiome has arrived, recent studies have revealed that organisms are not sterile entities but biological unions that are physiologically and metabolically associated with microbial communities. The bacterial microbiome has been a research focus in the past decade because of the easiness of experiments and analyses. However, more and more studies are including the mycobiome (the fungal part of the microbiome) due to its impact on host health. We have examined fungal communities associated with rice plants and humans for the last five years. From the works, we revealed veiled evolutionary relationships between rice and its associated microbial communities and the significance of the fungal communities as a proxy of dysbiosis. Thus, mycobiomes will expand our knowledge about the nature of microbiomes associated with hosts and environments.

S3-4

Analysis of Human Gut Mycobiome: Culturomics and Sequencing Approaches

Piyapat Rintarhat[1], Yong-Joon Cho[2*], and Won Hee Jung[1]

[1]Department of Systems Biotechnology, Chung-Ang University,
[2]Department of Molecular Bioscience, Kangwon National University

The human gut is colonized by diverse microorganisms, including bacteria, viruses, protozoa, and fungi. Several studies have suggested that the gut fungal microbiome (mycobiome) impacts host immunity and the development and progression of human diseases. However, most gut microbiome studies have focused exclusively on bacteria, and the mycobiome in the organ has largely been unexplored. Here, we developed a culturomics platform to isolate the fungal strains from fecal samples of a cohort of patients with ulcerative colitis (UC) and compared them with those of healthy subjects (HT). Moreover, we optimized the methodology for amplicon sequencing analysis to compare the fungal community structure between the UC and HT subjects. Our culturomics analysis showed that, overall, most identified fungal colonies belonged to the phylum Ascomycota followed by Basidiomycota both in HT and UC. The total number of the colonies from the fecal samples of UC was significantly higher than that of HT, suggesting that more fungal strains may persist in the intestine of UC compared to that of HT. Finally, we will present the results of the comparisons between different methodologies for amplicon sequencing analysis for the fungal community analysis. Our study emphasizes the importance of the gut mycobiome and provides useful information on human mycobiome analysis.

[Supported by the National Research Foundation of Korea (NRF)]

Poster

A001

Development of the *In Vitro* Platform Techniques for Investigating Bacteria-Fungi Interaction in Gut Microbiome Using *Candida albicans* and *Escherichia coli*

Yujin Lee, Ji-Young Lee, Seong-Ryong Yu, Soojin Yu, Dong-Woo Lee, and Yong-Sun Bahn*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

The human gut microbiome is closely related to human health and disease state. However, there are not many studies on the interaction between fungi and bacteria comprising the gut microbiota and their effects on the host. Hence, we aimed to develop *in vitro* platform techniques for investigating bacteria-fungi interaction using *Candida albicans* and *Escherichia coli* as model organisms, which are common intestinal bacteria and fungus. To co-culture these microbes, we selected 0.5x blood-heart-infusion (BHI) media as a culture media and perform co-culture under anaerobic condition at 37°C, to simulate intestinal environment. We analyzed the transcriptional profiling changes occurring during co-incubation. To this end, we collected mono-culture cells of both *C. albicans* and *E. coli* as control samples and co-cultured cells were collected at 1, 6, and 24 h after co-culture, and isolated total RNA using Trizol method. Then, RNA-seq was performed, and the result was analyzed by comparative analysis. The DEG analysis indicates that expression regulation pattern of the 1 h co-culture sample was most dynamic in *E. coli* but the gene expression of *C. albicans* was decreased during co-culture. KEGG analysis revealed that *C. albicans* and *E. coli* had similar terms such as metabolic pathways, carbon metabolism and biosynthesis of secondary metabolites. In future studies, we will optimize experimental methods for multi-omics analysis of bacteria-fungi interaction.

[Supported by grants from NRF]

A002

Multi-omics Profiling Reveals New Pathways Regulating Hyphal Morphogenesis in *Candida albicans*

Kyunghun Min[1,2], Thomas F. Jannace[1,3], Haoyu Si[1,4], Krishna R. Veeramah[5], John D. Haley[6,7], and James B. Konopka[1*]

[1]Department of Microbiology and Immunology, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY, USA,

[2]Present address: Department of Agricultural Biotechnology, Seoul National University,

[3]Present address: Department of Quality Assurance, Biogen, Morrisville, NC, USA,

[4]Present address: School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA,

[5]Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA,

[6]Department of Pathology, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY, USA,

[7]Biological Mass Spectrometry Shared Resource, Renaissance School of Medicine, Stony Brook University (SUNY), Stony Brook, NY, USA

The human fungal pathogen *Candida albicans* switches between budding and filamentous hyphal morphologies to gain advantages for virulence and survival in the host. Although adenylyl cyclase has been thought to be a master regulator that controls this switch, we identified *C. albicans* pseudorevertant mutants that grow better and form hyphae in the absence of adenylyl cyclase and cAMP. The mutant cells were also able to induce hyphal-associated genes in the absence of cAMP that are needed for virulence. Integrating information from different omics approaches identified cAMP-independent mechanisms that promote hyphal growth. This includes phosphoproteomic studies that revealed key roles for the Cdc28 cyclin-dependent kinase and casein kinase 1 in promoting hyphal growth. In addition, integrating transcriptomic and proteomic data revealed that post-transcriptional mechanisms regulate the levels of a set of key transcription factors that are important for hyphal induction, suggesting a special type of translational regulation. These studies better define the pathways that stimulate *C. albicans* to switch from budding to hyphal growth, which is important for invasion into tissues, escape from the immune system, and biofilm formation.

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A003

Identification of the Calcineurin Pathway in Pan-drug-resistant Fungal Pathogen *Candida auris*

Hyunjin Cha, Ji-Seok Kim, and Yong-Sun Bahn*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Candida auris is an emerging pan-drug-resistant fungal pathogen. Even though candidiasis exhibits high mortality rate as *C. auris* is resistant to multiple antifungal drugs, signaling pathways in *C. auris* are largely yet unknown. Therefore, we evaluated the pathobiological roles of the calcineurin pathway, which is known to be essential for virulence and drug resistance in other fungal pathogens, in *C. auris*. Calcineurin is a calcium and calmodulin-dependent serine/threonine protein phosphatase. Here, we showed that the catalytic subunit of calcineurin, *CNA1*, and the regulatory subunit of calcineurin, *CNB1*, have multiple roles in cell wall/membrane-damaging stress responses and antifungal drug resistance. Moreover, we observed that the knock-out mutant of *CRZ1*, a transcription factor of calcineurin, showed generally similar phenotypes as *cna1*Δ and *cnb1*Δ but at reduced severity. However, *crz1*Δ presented resistance against echinocandin class of drugs, while *cna1*Δ and *cnb1*Δ displayed sensitivity. Additionally, we constructed a knock-out mutant of *CRZ2*, a putative calcineurin-related transcription factor, but the mutant did not display the phenotypes related to calcineurin pathway, indicating that Crz1, but not Crz2, is likely to be a transcription factor downstream of calcineurin pathway. In conclusion, the calcineurin pathway plays pivotal roles in maintaining cell wall/membrane integrity and conferring resistance to various antifungal drugs in pan-drug-resistant *C. auris*.

A004

Identification and Functional Characterization of EHT1 and EAT1, Encoding Alcohol Acyltransferases for Aroma Generation, in *Wickerhamomyces subpelliculosus*

Hyeon Jin Kim, Yong Uk Cho, Hye Yun Moon, and Hyun Ah Kang*

Department of Life Science, Chung-Ang University

Aroma ester components, produced via alcohol acyltransferase (AAT)-catalyzed reactions, are responsible for flavor in fermented foods. From the *de novo* whole genome sequencing data of *Wickerhamomyces subpelliculous* CBS5767^{T}, we identified five homologs of *ATF* encoding alcohol *O*-acetyltransferases with AATase Pfam domain, along with homologs of *EHT1* and *EAT1* encoding alcohol acyltransferases carrying α/β hydrolase fold domain and the Ser-Asp/Glu-His catalytic triad. Whereas the expression of the *WsATF* genes in the heterologous host *Saccharomyces cerevisiae* did not increase the levels of aroma volatile esters, the expression of *WsEHT1* and *WsEAT1* increased the levels of ethyl decanoate and ethyl acetate, respectively. Using green fluorescence protein fusion analysis, WsEat1p was shown to localize at mitochondria, as indicated by the presence of mitochondrial localization sequence (MLS) at its N-terminus, whereas WsEht1p at endoplasmic reticulum and lipid droplets, respectively. Notably, by removing MLS at the N-terminus, the expression level of WsEat1p in *E. coli* was significantly increased. Our results would provide an in-depth knowledge on formation of volatile aroma esters by *W. subpelliculosus*, a potential flavor-formers, along with new genetic sources, such as *WsEAT1*, useful for developing microbial cell factories for production of ethyl acetate, which can be also used as a biodegradable organic solvent.

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A005

The Interkingdom Interaction with *Staphylococcus* Influences the Antifungal Susceptibility of the Cutaneous Fungus *Malassezia*

Juan Yang and Won Hee Jung*

Department of Systems Biotechnology, Chung-Ang University

The skin is a dynamic ecosystem, and diverse microbes reside in this environment. The interaction between microbial species in the skin microbiota is thought to influence the health and disease of the skin although the roles of the intra- or interkingdom interactions remain to be elucidated. The interactions between *Malassezia* and *Staphylococcus*, which are the most dominant fungal and bacterial genera in the skin microbiota, respectively, have gathered attention. This study investigated how the interaction between *Malassezia* and *Staphylococcus* affects the antifungal susceptibility of the fungus to the azole antifungal drug ketoconazole. The susceptibility was significantly decreased when *Malassezia* was co-cultured with *Staphylococcus*. We found that acidification of the environment by organic acids produced from *Staphylococcus* mainly influenced the decrease of the ketoconazole susceptibility of *M. restricta* in the co-culturing condition. Furthermore, our data demonstrate that the significant increase of the ergosterol content and cell membrane/wall thickness of the *M. restricta* cells growing under the acidic environment may be the main cause of the altered azole susceptibility of the fungus. Overall, our study suggests that the interaction between *Malassezia* and *Staphylococcus* influences the antifungal susceptibility of the fungus and that pH has a critical role in the polymicrobial interaction in the skin environment.

[Supported by grants from NRF.]

A006

Novel Plant-specific Nuclear Localization Sequence of MoHTR1, a Nuclear Effector of the Rice Blast Fungus, is Crucial for Translocation to Rice Nucleus and Immune-response by Transcriptional Reprograming

You-Jin Lim[1], Hyunjun Lee[2], Yoon-Ju Yoon[2], Gobong Choi[3], Seongbeom Kim[2], and Yong-Hwan Lee[1,2,3]*

[1]Research Institute of Agriculture and Life Sciences, Seoul National University,
[2]Department of Agricultural Biotechnology, Seoul National University,
[3]Interdisciplinary Program in Agricultural Genomics, Seoul National University

Plant pathogens secrete effectors to modulate the host immune system. Nuclear effectors translocated in the host nuclei and interact with proteins and DNA to regulate various defense mechanisms. Nuclear localization sequence (NLS) is the most well-known factor in transporting proteins from cytoplasm to nucleus. However, molecular mechanism on NLS-associated transportation and the roles of NLS in transcriptional reprograming are not well understood. We previously reported that MoHTR1, a nuclear effector of the *Magnaporthe oryzae*, is translocated to rice nuclei but not in fungal nuclei. We found one NLS (PGRSKKE) in MoHTR1 by site-directed mutagenesis. We further identified that RxKK residues was important for the nuclear localization of MoHTR1. MoHTR1 NLS altered the localization of cytoplasmic effectors of *M. oryzae* in the host. Furthermore, nuclear effector candidates which have RxKK sequence were also localized in rice nuclei. SUMOylation, post-translational modification, was involved in the secretion and translocation of MoHTR1 to biotrophic interfacial complexes and host nuclei. In addition, MoHTR1 NLS was important for pathogenicity of *M. oryzae* by reprograming of defense-related genes and was associated with transcriptional regulation of host target gene candidates. Taken together, our findings will provide unprecedented details the roles of plant-specific NLS on nuclear effector in pathogen-host interactions.

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A007

Genome-wide Functional Analysis of Canonical WD40 Repeated Proteins in *Cryptococcus neoformans*

Jin-Tae Choi, Seok-Hwan Jung, Hyunjin Cha, and Yong-Sun Bahn*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Cryptococcus neoformans is one of the major human fungal pathogens which causes death by meningoencephalitis. The cell signaling regulation in various environments and host conditions is known to be important for the survival and virulence of *C. neoformans*. Among the cell signaling regulation, protein-protein interaction (PPI) is known as an essential phenomenon. Though the WD40 domain is one of the most common and abundant domains related to PPIs, the role of WD40 domain-containing proteins remains elusive. We focused on 94 canonical WD40 domain-containing genes, and constructed knockout mutants and examined their *in vitro* phenotypic traits under 30 different growth conditions. For the genes which could not delete, we replaced the promoter to conditionally regulate the expression of target genes. As a result, we discovered Rav1, which known as a subunit of the regulator of ATPase of vacuoles and endosomes (RAVE) complex in the model yeast, was related to cellular growth on various temperature and stress conditions and the production of virulence factors in *C. neoformans*. Through further study, we can dissect the roles of WD40 proteins and PPI partners on various stress responses and host interaction of *C. neoformans*, and these findings will provide comprehensive insight to develop the PPI inhibitors as novel antifungal agents.

A008

Uncovering the Signaling Networks of a PP2A-like Phosphatase Sit4 Required for Brain Infection of *Cryptococcus neoformans*

Soojin Yu, Yeseul Choi, Yujin Lee, and Yong-Sun Bahn*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Phosphatases play critical roles in regulating cellular signaling networks involved in the survival and virulence of fungal pathogens. In this study, we aim to unravel the signaling networks of a protein phosphatase 2A (PP2A)-like phosphatase *SIT4* in *Cryptococcus neoformans*, an opportunistic fungal pathogen that causes fatal meningoencephalitis. From our previous study, we have identified *SIT4* as a virulence-related phosphatase that promotes blood-brain barrier adhesion and crossing. To elucidate the factors involved in the regulation of *SIT4*, a red-fluorescent fusion protein was constructed for pull-down assay, and one putative regulatory subunit, *SAP190* (*SIT4*-associating protein 190), was identified. As *SIT4* is downstream of the TOR (target of rapamycin) pathway, both *sit4*△ and *sap190*△ displayed increased susceptibility against rapamycin. The loss of the *SAP190* gene also showed reduced BBB crossing, while virulence was not affected in the insect model. Moreover, because the TOR pathway regulates metabolic status, the expression of *SIT4* and *SAP190* under glucose starvation condition was observed. As a result, the expression of both *SIT4* and *SAP190* increased in the wild type strain under glucose starvation. Surprisingly, in basal condition, *SIT4* transcription increased in *sap190*△ while *SAP190* transcription increased in *sit4*△ at a level higher than the wild type. From here, we aim to identify the signaling networks of *SIT4* to uncover its role and mechanism in brain infection.

A009

Understanding Molecular Mechanisms of Zearalenone Production

Nahyun Lee[1] and Hokyoung Son[1,2]*

[1]*Department of Agricultural Biotechnology, Seoul National University,*
[2]*Research Institute of Agriculture and Life Sciences, Seoul National University*

Zearalenone (ZEA) is a polyketide mycotoxin produced by several *Fusarium* species. Three key enzymes, two polyketide synthase (Pks4 and Pks13) and an alcohol oxidase (Zeb1), are involved in the biosynthesis of ZEA by sequential reactions. However, other genes or molecular mechanisms interact with *ZEB2* during ZEA production have not been studied yet. To investigate genes interacting with ZEB2, wild type, *ZEB2* overexpressing, and *zeb2* deletion mutant strains were selected for RNA-sequencing analysis. I selected 23 genes which were either up-regulated in the *ZEB2* overexpressing strain and down-regulated in the *zeb2* deletion strain, which are potentially Zeb2 regulons. Among 23 ZEA regulated (*ZR*) genes, only *zr32* showed reduced ZEA production compared to the wild type. Because *ZR32* was annotated as nonribosomal peptide synthetase 15 (NRPS15)-encoding gene, I have been functionally characterizing *ZR32* to investigate unknown mechanisms of the gene in ZEA biosynthesis. To identify mechanisms underlying direct regulation of Zeb2, protein binding microarray (PBM) and electrophoretic mobility shift assay (EMSA) were performed to discover consensus binding sequences of Zeb2. These results will reveal regulatory mechanisms of Zeb2 and enhance our knowledge of the molecular mechanisms of secondary metabolite production including ZEA.

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A010

Exploring and Exploiting of Sphingolipid Biosynthesis Pathway in *Yarrowia ipolytica* for Production of Human-type Sphingoid Bases and Ceramides

Seo Hyeon Shin, Hye Yun Moon, Hae Eun Park, and Hyun Ah Kang*

Department of Life Science, Chung-Ang University

The biosynthesis of sphingolipid begins with the condensation of L-serine and palmitoyl-CoA to yield the C18 carbon unit 3-ketosphinganine, which is reduced to yield sphinganine. In this study, to block the fungal specific phytosphingosine-based sphingolipid biosynthesis pathway in the oleaginous yeast *Yarrowia lipolytica*, the *SUR2* gene, encoding sphinganine C4-hydroxylase responsible for conversion of sphinganine to phytosphingosine, was disrupted. The resultant *Y. lipolytica sur2* null mutant (*Ylsur2*Δ) exhibited retarded growth with increased pseudohyphal formation and displayed increased sensitivity to high temperature, osmotic, and cell wall stresses compared to the wild-type strain. Notably, the *Ylsur2*Δ mutant showed increased production of sphinganine, which was mostly secreted to the cell surface even without acetylation. Subsequent disruption of the *SLD1* gene, encoding fungal specific Δ8 sphingolipid desaturase, partly rescued the growth defect of *Ylsur2*Δ by recovering yeast-type growth and increased production of human-type glucosylceramides. Additional introduction of mouse ceramidase into the *Ylsur2*Δ*sld1*Δ double null mutant led to the significantly increased production of sphinganine and sphingosine. Our results present the high potential of the engineered *Y. lipolytica* strains as hosts for the secretory production of human-type sphingoid bases and ceramides, which are useful ingredients for cosmeceutical or nutraceutical formulations.

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A011

Characterization of *fphA*, *lreA* and *lreB* Genes in *Aspergillus flavus*

Hye-Min Park[1], Ye-Eun Son[1], and Hee-Soo Park[1,2*]

[1]School of Food Science and Biotechnology, Kyungpook National University,
[2]Department of Integrative Biology, Kyungpook National University

Aspergillus flavus is one of the potent pathogenic fungi that produce carcinogenic secondary metabolites, commonly known as aflatoxins. *A. flavus* mainly spread through airborne asexual spores, which produced by asexual development under the influence of environmental factors such as light, temperature and aeration. Light is a general environmental factor that regulates asexual development, stress resistance, and even mycotoxins in several fungi. When light is served as an external signal, photoreceptor proteins sense through a chromophore. Previous studies showed that FphA is a phytochrome for red-light detection, and LreA and LreB are white collar homologs for blue-light detection in *Aspergillus nidulans*. It was proved that they are essential for asexual development in *A. nidulans*. But it has not yet been studied in *A. flavus.* Therefore, we have studied the roles of light sensors in *A. flavus*. First, we produced each deletion mutant and analyzed asexual development. In results, the number of asexual spores was decreased in the deletion of *lreA* and *lreB*, while there was not affected in the *ΔfphA* mutant. Next, we checked the aflatoxin production in each deletion strain. As a result, light sensors were found to affect the production of aflatoxins. Taken together, these results suggest that three photosensors are present in *A. flavus* and regulate asexual development and aflatoxin production.

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A012

A myb-like protein A, MylA, is Indispensable for Fungal Growth, Development, and Stress Tolerance in *Aspergillus* Species

Ye-Eun Son[1], He-Jin Cho[1], Hye-Min Park[1], and Hee-Soo Park[1,2]*

[1]School of Food Science and Biotechnology, Kyungpook National University,
[2]Department of Integrative Biology, Kyungpook National University

The representative filamentous fungi, *Aspergillus*, proliferate by forming conidia (asexual spores), and the procedures of making conidia (conidiation) are regulated by a variety of transcription factors (TFs). Myb-like transcription factors are one of the largest TF families that modulate the levels of transcription or translation of their targets by sequence-specific DNA binding activities and protein-protein interaction activities. However, the role of Myb-like TFs is not completely understood in *Aspergillus* species. Therefore, we identified 7 Myb-like (Myl) proteins that are highly expressed in the *Aspergillus nidulans* conidia. To examine their roles in *A. nidulans*, each deletion mutant (*mylA~F*) was generated. Among them, the deletion of *mylA* showed defective fungal growth and asexual development. Also, the trehalose content, conidial viability, and stress tolerance decreased in *mylA* null mutant conidia. The additional transcriptomic analysis supported that MylA contributes to fungal growth, development, and conidial stress tolerance. Furthermore, we studied whether the functions of MylA were conserved in *Aspergillus flavus* and *Aspergillus fumigatus*. As a result, deletion of *mylA* homolog showed abnormal fungal growth, conidiation, and reduced external stress tolerance in *A. flavus* and *A. fumigatus*. Taken together, these results suggest that MylA plays pivotal roles in the appropriate fungal growth, development, and external stress response in three *Aspergillus* species.

A013

A Novel Spore-specific Transcription Factor is Essential for Conidial Maturation and Dormancy in *Aspergillus* Species

Ye-Eun Son[1] and Hee-Soo Park[1,2*]

[1]School of Food Science and Biotechnology, Kyungpook National University,
[2]Department of Integrative Biology, Kyungpook National University

Aspergillus, a filamentous fungus that makes up the majority of airborne fungi, mainly propagates by forming asexual spores called conidia. Conidia formation is regulated by various transcription factors (TFs). We studied putative spore-specific TFs in three representatives *Aspergillus* based on transcriptomic analysis. As a result, twenty-two spore-specific TFs were identified, and each deletion mutant was phenotypically analyzed in *A. nidulans*. Among them, we characterized one of the spore-specific-C_2H_2 zinc finger A SscA. The *ΔsscA* mutant showed defective conidiation, conidial viability, and reduced stress tolerance in *A. nidulans*. The amount of trehalose in the *ΔsscA* mutant was decreased compared to that of the WT and deletion of *sscA* caused induced germ tube formation. Furthermore, transcriptome data showed that deletion of *sscA* was involved in the response of conidia to stimuli and stress. The mRNA levels of the β-glucan biosynthesis gene and the sterigmatocystin gene cluster were upregulated in *sscA* mutant conidia. These were validated by the phenotypic analyses. In addition, we confirmed that the roles of SscA in conidia were conserved in *A. flavus* and *A. fumgiatus*. Taken together, these results suggest that SscA is a novel spore-specific transcription factor, essential for proper conidia formation, conidia maturation, conidia dormancy and secondary metabolites in *A. nidulans*. And the functions of SscA in conidia are conserved in three representative *Aspergillus* spp.

A014

Functional Analysis and Application of *Yarrowia lipolytica* Ceramidase, YlYdc1p, Involved in Sphingolipid Metabolism, for Improved Production of Sphingoid Bases

Eun-Joo Jeon, Seo Hyeon Shin, Hye Yun Moon, and Hyun Ah Kang*

Department of Life Science, Chung-Ang University

Ceramidase plays an important role in regulating ceramide levels by hydrolyzing ceramide, a central molecule in the pathway of sphingolipid metabolism. Here, we performed functional analysis of *Yarrowia lipolytica YDC1*, encoding a predicted protein with 36% and 35% identity to *Saccharomyces cerevisiae* ceramidases Ypc1p and Ydc1p, respectively. *Y. lipolytica* Ydc1 protein (YlYdc1p), composed of 320 amino acids, has a ceramidase domain and seven transmembrane segments. The *YlYDC1* mRNA level was shown to be increased upon shift to glycerol broth (GB) from YPD medium in qRT-PCR analysis. Despite the absence of an ER retention sequence, the GFP fusion analysis showed that YlYdc1p is localized to the ER membrane. The overexpression of *YlYDC1* in the *S. cerevisiae* wild-type (WT) and *ypc1*△ *ydc1*△ mutant strains generated the profile change of sphingoid long-chain bases (LCBs), the breakdown products of ceramides. Notably, the TLC-based LCB profiles by *YlYDC1* overexpression were more similar to those by *ScYPC1* overexpression compared to *ScYDC1* overexpression, regardless in WT and *ypc1*△ *ydc1*△ background. The increase of both dihydrosphigosine and phytosphingosine at comparable levels by *YlYDC1* overexpression strongly indicated that YlYdc1p can cleavage both dihydroceramide and phytoceramide without substrate preference. Our results present YlYdc1p as a manipulation target to increase sphingoid bases in this oleaginous yeast with high industrial potential.

[Supported by MOTIE(20008739)]

A015

Anaplerotic Roles of Pyruvate Carboxylase in Plant Pathogenic Fungi *Fusarium graminearum* and *F. oxysporum*

Soobin Shin[1], Heeji Moon[1], Hun Kim[2], Gyungja Choi[2], and Hokyoung Son[1,3]*

[1]Department of Agricultural Biotechnology, Seoul National University,
[2]Center for Eco-friendly New Materials, Korea Research Institute of Chemical Technology,
[3]Research Institute of Agriculture and Life Sciences, Seoul National University

Anaplerosis describes enzymatic reactions or pathways that replenish the pools of metabolic intermediates in the tricarboxylic acid (TCA) cycle. Pyruvate carboxylase (PC) has an important anaplerotic function by catalyzing the carboxylation of pyruvate to form oxaloacetate. Diverse biochemical roles of PC in different mammalian tissues have been well studied because anaplerosis contributes specifically to the production of biomass during tumor cell proliferation. However, the functions of PC have not been investigated in filamentous fungi including pathogenic fungi. Here, we characterized the molecular functions of PC in *Fusarium graminearum* and *F. oxysporum*, major fungal plant pathogens which have different pathosystems and therefore may utilize different carbon metabolism for pathogenesis. Surprisingly, the *pc* mutant of *F. oxysporum* resulted in pleiotropic defects in hyphal growth, conidiation, and virulence, while disruption of *PC* gene in *F. graminearum* did not affect virulence. In addition, the *pc* mutant exhibited defect in pectin utilization, activation of cell wall degrading enzymes (CWDEs), and *in vitro* extracellular polygalaturonase activity. Genes related with glucose repression were abnormally regulated in *pc* mutant under glucose supplemented condition. The results of this study implied that the anaplerotic role of PC might differently affect development and pathogenesis in *Fusarium* spp. and that the function of PC is correlated with glucose repression.

A016

Evaluation of Dermatophyte Growth Inhibitory Effect of Multi-wavelength VCSEL Laser

Seun Kang[1], Soon-Young So[2], Jin Park[3], and Kyung-Tae Lee[1]*

[1]Korea Zoonosis Research Institute, Jeonbuk National University,

[2]Optowell Co.,Ltd.,

[3]Department of Dermatology, Jeonbuk National University Medical School

Athlete's foot is a disease caused by the dermatophyte which penetrates the stratum corneum. Onychomycosis, fungal nail infection, is the most common, accounting for about half of all athlete's foot. Existing athlete's foot treatments have been reported to have problems such as drug penetration, duration of effect, and side effects, so it is necessary to develop an appropriate treatment method that can be applied to the patients who are difficult to treat with existing drugs. In this study, low-power laser (1~500 mW) was applied for the treatment of onychomycosis to evaluate the growth inhibitory effect of Trichophyton and Trichophytes, two major dermatophytes. In conclusion, the multi-wavelength VCSEL laser alone inhibited the growth of dermatophytes. Compared to antifungal agent alone, when used in combination with multi-wavelength VSCEL laser, it was confirmed that not only the antifungal effect was increased, but also the sustained effect was excellent. It presents the potential as an effective and safe treatment device to treat dermatophytes either alone or in combination with antifungal agents.

A017

Application of Direct PCR for Identification and Phylogenetic Analysis of Fungal Species

Hosung Jeon[1], Jung-Eun Kim[2,3], Jung-Wook Yang[4], Hokyoung Son[1,2*], and Kyunghun Min[1,2*]

[1]Department of Agricultural Biotechnology, Seoul National University,
[2]Research Institute of Agriculture and Life Sciences, Seoul National University,
[3]Research Institute of Climate Change and Agriculture, National Institute of Horticultural and Herbal Science, Rural Development Administration,
[4]Crop Cultivation and Environment Research Division, National Institute of Crop Science, Rural Development Administration

Precise and rapid detection of fungal pathogens is essential for effective disease management. Sequencing universal DNA barcodes has become the standard method for the diagnosis of fungal diseases, as well as for identification and phylogenetic analysis. A major bottleneck in obtaining DNA sequence data was the laborious and time-consuming process of sample preparation for genomic DNA. Here, we describe a direct PCR approach that bypasses the DNA extraction steps to streamline the molecular identification of fungal species. Using a direct PCR approach, we successfully sequenced the nuclear ribosomal internal transcribed spacer (ITS) region for the representatives of major fungal lineages. To demonstrate the usefulness of this approach, we performed a phylogenetic analysis of the *Fusarium fujikuroi* species complex, which causes bakanae disease of rice and mycotoxin contamination. A total of 28 candidate strains were isolated from rice seeds in the Republic of Korea, and the identity of the isolates was determined using the DNA sequence of both ITS and translation elongation factor 1-α regions. In addition, 17 *F. fujikuroi* isolates were examined for fumonisin (FB) production in rice medium using an ELISA. Phylogenetic and toxigenic analyses showed that the *F. fujikuroi* strains could be divided into two groups: FB producers and non-producers. These results will accelerate the molecular identification of fungal pathogens and facilitate the effective management of fungal diseases.

A018

Omics-based Characterization and CRISPR/CAS9-based Genome Editing Tool Development of *Saccharomyces cerevisiae* Industrial Strains

Ye Ji Son, Min-Seung Jeon, Jong Beom Kim, Hye Yun Moon, Seong-il Eyun, Seung Hwan Lee, and Hyun Ah Kang*

Department of Life Science, Chung-Ang University

The industrial potential of *S. cerevisiae* has extended beyond its traditional use in food fermentation into various healthcare sectors, such as in the production of therapeutic recombinant proteins. In this study, comparative genomics analysis was carried out with three industrial strains of *S. cerevisiae*, Y98-5, KSD-YC, and Y2805. Notably, the genomes of Y98-5 and KSD-YC, the starter strains for commercial rice wine production in Korea, were revealed as heterozygous diploid, whereas that of Y2805, a host for recombinant protein production, was haploid. Genome-based phylogenetic analysis indicated that Y2805 was closely associated with the reference strain S288C, whereas KSD-YC and Y98-5 were grouped with Asian and European wine strains, respectively. Phenotype microarray (PM) analysis further showed that KSD-YC and Y98-5 displayed broader substrate utilization than S288C and Y2805. By integrating with the genomic data, SNPs were mapped in the genes responsible for the observed differences in the PM data. Furthermore, we developed genome editing tools for the diploid industrial *S. cerevisiae* strains via two approaches, a plasmid-mediated and a ribonucleoprotein-mediated Cas9-gRNA gene editing. Our omics-based analysis elucidated the evolutionary history with genetic and metabolic diversity of industrial *S. cerevisiae* strains. The CRISPR-based tools are expected to establish an important foundation for genetic manipulation of diploid industrial host cells.

A019

Isolation of Drug-resistant Strains and Identification of Drug-resistance Regulatory Signaling Networks

Eui-Sung Kim[1,2] and Kyung-Tae Lee[1*]

[1]Korea Zoonosis Research Institute, Jeonbuk National University,
[2]Division of Life Science, Jeonbuk National University

In the concept of One-Health, the most important medium that connects our surroundings and the natural environment is wild animals, through which many zoonotic diseases can spread. In particular, it has been reported that drug resistance of microorganisms can be developed by anti-microbial agents sprayed in large quantities on farmland and can be spread by the wild animals. The opportunistic infections by microorganisms with drug resistance may increase as these strains are introduced into cities. In this study, the medium connecting the environment, animals, and humans was assumed to be animals with relatively high body temperature. The experiment is to compare and analyze the degree of drug resistance of these strains with reference strains and clinical strains by collecting high-temperature-resistant fungal strains from hospital patients or from animal or bird feces in the environment. In the samples, it was possible to selectively classify fungi under the acidic and high-temperature culture conditions, where bacteria are difficult to reproduce. It is possible to isolate and identify fungi, and to measure susceptibility to commercially available antifungal agents. The obtained strains can be comparatively analyzed according to the collection location and can be provided as a database that can comprehensively predict the distribution and migration route of antifungal resistance-related genes.

A020

Unraveling the Essential Transcription Factors and Their Roles in *Cryptococcus neoformans*

Seung-Heon Lee[1], Yu-Byeong Jang[1], Jin-Tae Choi[1], Kyung-Tae Lee[1], Alexander Idnurm[2], and Yong-Sun Bahn[1*]

[1]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University,
[2]School of BioSciences, the University of Melbourne, VIC3010, Australia

C. neoformans causes cryptococcosis, which is one of the leading causes of death among HIV patients. Therefore, developing antifungal drugs is crucial. Essential proteins can be notable as they are required for growth therefore considered as targets. In this study, we aim to identify essential TFs of *C. neoformans* and characterize their roles. Our previous research revealed that 23 genes could be essential TFs in the fungus because they cannot be deleted. We constructed conditional expression strains for the 19 TFs by replacing their promoters with the copper-regulated *CTR4* promoter. Under repressive condition, conditional expression strains of 11 TFs showed defects, implying that these TFs are required for growth. To verify their essentiality for the viability of the fungus, we constructed heterozygous mutants with diploid strain using the drug resistance marker, and are currently performing spore analysis. After harvesting spores from the heterozygous mutant, we analyze the genotypes of progenies. If we could get spores with the drug resistance, we would classify the target genes as non-essential. On the other hand, if we were unable to get spores with the drug resistance, we would regard the genes as essential and study their functions with overexpression strains. By discovering essential TFs and their traits, we are expecting not only to broaden our understanding of TF networks in *C. neoformans*, but also to suggest potential targets for developing antifungal agents.

A021

Characterization of Cell Wall Proteome in *Fusarium graminearum*

Heeji Moon[1], Jessica Winarto[2], Soobin Shin[1], Hosung Jeon[1], Kyunghun Min[1], Dae-Geun Song[2], and Hokyoung Son[1,3]*

[1]Department of Agricultural Biotechnology, Seoul National University,
[2]Natural Product Informatics Research Center, Korea Institute of Science and Technology (KIST) Gangneung Institute of Natural Products,
[3]Research Institute of Agriculture and Life Sciences, Seoul National University

Fusarium head blight, caused by the plant pathogenic fungus *Fusarium graminearum* is a devastating disease of wheat, barley, and maize worldwide. It causes severe yield losses and contaminates grains by accumulating mycotoxins which are harmful to animal health. The fungal cell wall forms the interface between the fungus and the host plant. Especially, cell wall proteins (CWPs) are expected to play an important role in virulence. However, little is known about CWPs involved in virulence in plant pathogenic fungi. To characterize the cell wall subproteome of *F. graminearum*, we performed LC-MS/MS proteomic analyses using enhanced CWP extraction procedures which reduce non-CWPs contamination, and 616 putative CWPs were enriched. Among them, 165 “typical” CWPs predicted to contain a signal peptide, the glycosylphosphatidylinositol (GPI) motif, and/or to localized at extracellular were identified. A combined proteome and transcriptome analysis was applied to identify “typical” CWPs involved in plant infection. CWPs were clustered according to their temporal expression profile during plant infection and five gene clusters that were specifically expressed in each infection stage were identified. To elucidate the underlying mechanisms of CWP-mediated virulence, 20 CWPs candidates were selected. Further study will focus on the elucidation of the roles of virulence-related CWPs. This study provides a comprehensive insight into the pathobiological CWPs of plant pathogenic fungi.

A022

Deciphering the Cryptic Function of Mitogen-activated Protein Kinases Cpk2 and Mpk2 in *Cryptococcus neoformans*

Yu-Byeong Jang and Yong-Sun Bahn*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

In basidiomycetous human fungal pathogen, *Cryptococcus neoformans*, has five mitogen-activated protein kinases (MAPKs) play central roles in various functions. Apart from three major MAPKs, *C. neoformans* has Cpk2 and Mpk2, which are paralogs of Cpk1 and Mpk1, but their roles remain elusive. Our previous genome-wide functional analysis revealed that Cpk2 plays minor roles in osmotic and genotoxic stress response and melanin production but is dispensable for mating process unlike Cpk1. Lack of *CPK2* does not lead to defects in virulence of *C. neoformans*. Similarly, unlike Mpk1, Mpk2 plays minor roles in membrane stability, antifungal drug resistance, and melanin, urease production. Mpk2 is essential for virulence in mouse infection as well as its paralog Mpk1. In this study, we aimed to elucidate the functional connection of Cpk2 and Mpk2 to Cpk1- and Mpk1-dependent signaling pathways. Overexpression of *CPK2* could restore the mating defect of *cpk1* Δ, including mating pheromone production, filamentation, and sporulation. Cpk2 was regulated in Mat2-dependent manner, which is known as a downstream transcription factor of the Cpk1 mating pathway. Then, overexpression of *MPK2* could also partially restore the growth defect of *mpk1* Δ under cell wall stress as well as restore the basal urease production level. Moreover, *mpk1* Δ *mpk2* Δ displayed more drastic defect in melanin production compared to *mpk1* Δ, which is a key virulence factor of the *C. neoformans*.

A023

Uncovering Genetic Network of *In Planta* Specific Expression of a Nuclear Effector Gene, *MoHTR1*, of the Rice Blast Fungus

Yoon-Ju Yoon[1], You-Jin Lim[2], Seongbeom Kim[1], Hyunjung Chung[1], and Yong-Hwan Lee[1,2,3*]

[1]Department of Agricultural Biotechnology, Seoul National University,
[2]Research Institute of Agriculture and Life Sciences, Seoul National University,
[3]Center for Plant Microbiome Research, Seoul National University

During the host-pathogen interactions, fungi secrete effector proteins that disrupt the host immune system for successful infection. However, there are only limited reports about the mechanisms of regulating *in planta* expression of effector genes. To understand the regulation mechanism of *MoHTR1*, we performed a serial deletion experiment in the promoter region of *MoHTR1* with sGFP tagging, and found 8 bp of *cis*-elements (TATTTCGT) for *in planta* specific expression. Transversion substitution mutation of these 8-bp sequences led to reduced virulence of the fungal pathogen equivalent to the deletion of *MoHTR1*. Furthermore, we discovered that *in planta* specific expression of *Slp1* is regulated by the same *cis*-element of *MoHTR1*. To examine whether *MoHTR1*'s promoter can regulate expressions of other genes, we performed the promoter switching using *MobZIP14*, a transcriptional factor gene expressing during vegetative growth but not in the infection stage. We found that *MobZIP14* was expressed during the infection stage with the promoter of *MoHTR1*. Our findings will provide comprehensive insights into the regulatory mechanisms of *in planta* specific expression of fungal effector genes in the rice blast fungus.

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A024

Structured Study of Host-derived Cues for the Regulation of Pathogenicity-associated Transcription Factors in *Cryptococcus neoformans*

Seong-Ryong Yu, Minjae Lee, Kyung-Tae Lee, and Yong-Sun Bahn*

Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University

Cryptococcus neoformans is a causative agent of global fungal meningoencephalitis, which results in more than 180,000 deaths annually. However, its treatment option is limited mainly due to a lack of complete understanding of how the pathogen interacts with the host during infection and disease progression. To analyze this, *in vivo* transcription profiling was performed to monitor 180 transcription factors (TFs) during the infection process. Here, we focused on 12 TFs that strongly induced in HMC. To classify which host factor causes the induction of genes during infection, HMC signals were dissected into temperature, carbon, and nitrogen starvation. Notably, we found that three distinct cues made a significant contribution to the regulation of their expression. The expression of six genes was markedly induced by temperature upshift. Also, the expression of six genes was highly induced by glucose starvation, and nine genes were highly induced by nitrogen starvation. Deletion of *MLN1* caused growth defects supplemented with maltose or ammonium sulfate in a nutrient starvation medium, and transcriptome data of *mln1*Δ at the carbon starvation implied that *MLN1* related to the L-leucine degradation pathway. In conclusion, we systematically dissected host-signaling cues that affect *in vivo* expression of pathogenicity-related TFs, providing further insight into complex signaling pathways modulating the host-pathogen interactions of *C. neoformans.*

A025

Combination of Culture-dependent and -independent Methods to Unveil Hidden Biotic Interactions between *Tricholoma matsutake* and Soil Microbiome

Su-Min Kim[1], In-Hyup Bae[1], Hyun Kim[1,2], and Yong-Hwan Lee[1,2,3]*

[1]Department of Agricultural Biotechnology, Seoul National University,
[2]Research Institute of Agriculture and Life Sciences, Seoul National University,
[3]Interdisciplinary Program in Agricultural Genomics, Seoul National University

Tricholoma matsutake (Pine mushroom) is a valuable crop in Eastern countries because of its unique aromatic odor. The harvest amount of *T. matsutake* has decreased over decades due to habitat losses and climate change. Although the conditions of *T. matsutake* to grow in nature and the symbiotic relationship between *T. matsutake* and pine trees (*Pinus densiflora*) were discovered during last decade, the human-mediated growth of *T. matsutake* is still somehow unavailable to succeed. This study focuses on finding the missing elements between *T. matsutake* and surrounding soil microbes. Two different types of soil (Tm+ and Tm-) were collected from the *T. matsutake* habitat and analyzed by two different methods. Culture-dependent methods to study microbiomes in collaboration with culture-independent methods are expected to find the new biotic interactions between *T. matsutake* and soil microbiome.

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A026

The Casein Kinase 2 Complex Regulates the Growth, Differentiation, Stress Responses, and the Pathogenicity of *Cryptococcus neoformans*

Yeseul Choi[1], Seong-Ryong Yu[1], Ann-Yae Na[2], Sangkyu Lee[2], and Yong-Sun Bahn[1*]

[1]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University,
[2]College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University

The basidiomycete human fungal pathogen *Cryptococcus neoformans* causes fatal meningoencephalitis. However, the therapeutic options for treatment are currently highly limited. As a potential antifungal drug target, kinases have been considered to be good candidates as some of them play critical roles in virulence of pathogens. In our previous studies, we demonstrated that Cka1, which is a serine/threonine kinase and the catalytic subunit of the casein kinase 2 (CK2) complex. In this study, we aim to figure out the regulatory mechanism of the CK2 complex in *C. neoformans*. The cryptococcal CK2 complex consists of Cka1 and two regulatory subunits, Ckb1 and Ckb2. The *ckb1*Δ, *ckb2*Δ, and *ckb1*Δ *ckb2*Δ mutants exhibited increased susceptibility to antifungal drugs, oxidative stress, and DNA damaging agents. Notably, however, the *cka1*Δ *ckb1*Δ *ckb2*Δ mutants showed more severe growth defects and greater stress susceptibility than the *cka1*Δ mutants, indicating that the regulatory subunits may have Cka1-independent functions. Supporting this, we found that the CK2 complex is required for maintaining normal cell cycle and morphology. Considering pleiotropic roles of the CK2 complex in *C. neoformans*, we elucidated its downstream effector genes and proteins through transcriptomics and phosphoproteomics analyses, respectively. In conclusion, this study provides a comprehensive insight into the function and regulatory mechanism of the fungal CK2 complex.

A027

Comparative Analysis of Anticancer and Antibacterial Activities among Seven Species belong to the Genus *Trametes*

Ha Thi Kim Nguyen[1], Jiwon Lee[1], Yejin Park[1], Hyon Jin Park[1], Dong-Ku Kang[2], Jinho Kim[2], Soon Kil Ahn[1], Minkyeong Kim[3], Changmu Kim[3], and Jaehyuk Choi[1]*

[1]Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University,
[2]Department of Chemistry, College of Natural Sciences, Incheon National University,
[3]Microorganism Resources Division, National Institute of Biological Resources

Species in the genus *Trametes* (*Basidiomycota, Polyporales*) have been used in natural medicine for a long time. Many studies reported that mycelia or fruiting bodies of *Trametes* spp. exhibited effects of anticancer, antimicrobial, and anticoagulant activities. However, comparative analysis in this genus is scarce due to limitation of morphological identification and low sample number. In his study, the 19 Korean strains of seven *Trametes* species were chosen to generate a five-gene-based phylogeny with the 31 world-wide *Trametes* references. In addition, 39 culture extracts were prepared for 13 strains to test for anticancer and antibacterial activities. Strong anticancer activities were found in several extracts from *T. hirsuta* and *T. suaveolens*. Anticancer activities of *T. suaveolens*, *T.* cf. *junipericola* and *T. trogii* were first described here. The antibacterial ability of *T. versicolor* and *T. hirsuta* extracts has been confirmed. The antibacterial activities of *T. suaveolens* have been reported at the first time in this study. These results suggest an efficient application of the genus *Trametes* as the drug resources especially for anticancer agents.

A028

The Protein Phosphatase 2C Domain Contributes to the Pathobiological Function of Adenylyl Cyclase in *Cryptococcus neoformans*

Jae-Hyung Jin and Yong-Sun Bahn*

Yonsei University

The cAMP signaling pathway governs growth, differentiation, stress response and adaptation, and the pathogenicity of plant and animal fungal pathogens. The AC consists of multiple protein domains, including Ga-binding domain, Ras-associated domain, leucin-rich repeat, protein phosphatase 2C (PP2C), and AC-catalytic domain. Yet the function of the PP2C domain in AC remains unknown in most fungal pathogens. Here we functionally characterize the PP2C domain in the AC (Cac1) of *Cryptococcus neoformans*, which causes life-threatening human fungal meningoencephalitis in worldwide. To this end, we constructed *C. neoformans* strains containing $CAC1^{PP\triangle}$ and $CAC1^{AC\triangle}$ alleles, in which the PP2C and AC catalytic domains are deleted, respectively, and performed comparative phenotypic analysis in comparison with the wild-type and control strains. As expected, the AC catalytic domain is required for all the functions of Cac1 in *C. neoformans*: production of melanin and capsule, sexual differentiation, and stress response and adaptation. However, we found that the PP2C domain plays a partial role in melanin and capsule production, but is dispensable for stress response and adaptation. In conclusion, here we demonstrate that the PP2C domain contributes to the function of Cac1 in virulence factor regulation of *C. neoformans*.

A029

The Fusion Protein of SrbA Formed after Chromosomal Translocation Suppressed the Defect of SrbA Cleavage-activation in a *sec23-2* Mutant of *Aspergillus nidulans*

Jun-Yong Kwak and Suhn-Kee Chae*

Department of Life Science, Pai Chai University

Sterol element binding protein SrbA in *A. nidulans* is an essential transcription factor for survival in hypoxic environment. SrbA is anchored in ER due to its characteristic TM domain in normoxia. To switch to a transcriptionally active form in hypoxia, SrbA requires an elaborate cleavage process initiated by translocation from ER to Golgi where the 1st cleavage occurs. We have isolated many hypoxia-sensitive mutants in *A. nidulans* via forward genetics. Sec23 a component of the COPII complex responsible for the protein ER-to-Golgi transport was identified as one of the cognate genes among the hypoxia-sensitive mutants. The *sec23-2* mutants did not grow and failed to transport SrbA to Golgi in hypoxia. Suppressor mutants of *sec23-2* have been isolated and characterized. All of them except one were derived from reversions at the *sec23-2* mutation site. Interestingly, that exceptional suppressor mutant exhibited chromosomal translocation at the SrbA locus and produce a fusion protein of the SrbA N-terminus with the part of the hypothetical protein AN3143 on chromosome VI, thereby leading to cleavage-activation of SrbA in *sec23-2* mutant.

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Participants

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Participants in the 24th Korean Fungal Genetics & Biology Conference, 2023

강 민 영 (단국대학교)
강 현 아 (중앙대학교)
고 요 한 (전북대학교)
곽 준 용 (배재대학교)
권 순 학 (한국원자력연구원/연세대학교)
권 예 지 (전북대학교)
김 대 혁 (전북대학교)
김 성 환 (단국대학교)
김 수 림 (서울대학교)
김 수 민 (서울대학교)
김 신 일 (경상국립대학교)
김 예 인 (단국대학교)
김 유 경 (우석대학교)
김 의 성 (전북대학교)
김 종 범 (중앙대학교)
김 지 석 (연세대학교)
김 학 선 (우석대학교)
김 현 (서울대학교)
노 형 진 (단국대학교)
문 혜 연 (중앙대학교)
문 희 지 (서울대학교)
민 경 훈 (서울대학교)
박 민 욱 (전북대학교)
박 상 진 (우석대학교)
박 숙 영 (순천대학교)
박 용 순 (공주대학교)
박 윤 진 (공주대학교)
박 지 연 (서울대학교)
박 태 민 (공주대학교)
박 혜 민 (경북대학교)
박 희 수 (경북대학교)
반 용 선 (연세대학교)
배 인 협 (서울대학교)
상 현 규 (전남대학교)
손 도 은 (전남대학교)
손 문 일 (부산대학교)
손 예 은 (경북대학교)
손 호 경 (서울대학교)
신 서 현 (중앙대학교)
신 수 빈 (서울대학교)
양 주 안 (중앙대학교)
원 도 연 (연세대학교)
유 성 룡 (연세대학교)
유 수 진 (연세대학교)
윤 나 영 (단국대학교)
윤 윤 주 (서울대학교)
윤 진 호 (성신여자대학교)
이 강 찬 (서울대학교)
이 경 태 (전북대학교)
이 나 현 (서울대학교)
이 민 재 (서울대학교)
이 민 재 (우석대학교)
이 승 헌 (연세대학교)
이 용 환 (서울대학교)
이 유 진 (연세대학교)
이 정 훈 (서울대학교)
임 유 진 (서울대학교)
장 명 준 (공주대학교)
장 유 병 (연세대학교)
전 상 철 (우석대학교)
전 은 주 (중앙대학교)
전 지 선 (전북대학교)
전 호 성 (서울대학교)
정 광 우 (한국원자력연구원)
정 민 주 (전북대학교)
정 석 환 (연세대학교)
정 승 환 (경북대학교)
정 원 희 (중앙대학교)

정 인 희 (단국대학교)
조 용 욱 (중앙대학교)
조 준 우 (단국대학교)
조 현 욱 (단국대학교)
조 혜 진 (경북대학교)
지 수 영 (단국대학교)
진 재 형 (연세대학교)
차 현 진 (연세대학교)
채 순 기 (배재대학교)
최 선 규 (전남대학교)
최 소 형 (중앙대학교)
최 예 슬 (연세대학교)
최 재 혁 (인천대학교)
최 지 원 (서울대학교)
최 진 태 (연세대학교)
탁 은 정 (중앙대학교)
편 하 영 (고려비엔피)
한 갑 훈 (우석대학교)
한 지 우 (전북대학교)
홍 의 용 (공주대학교)

Piyapat Rintarhat (중앙대학교)
Siti Farzana Bazilah Bazla Hj Sa'adi (단국대학교)

(2019, The 22nd Korean Fungal Genetics and Biology Conference, Deoksan)

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제24회

진균유전생물 컨퍼런스