

제25회

진균유전생물 컨퍼런스

2024. 2. 1(목) ~ 2(금)

덕산 스피라스 리솜

The 25th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2024

주최

한국미생물학회 진균유전생물학 분과



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The Microbiological Society of Korea

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The Microbiological Society of Korea

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(사)한국미생물학회

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Timetable

| 2.1 (Thu) | 스테이타워 중연회장 SPACE B | |
|-------------|--------------------------------------|-----------------------|
| 12:30~13:10 | Registration | |
| 13:10~13:20 | Opening Ceremony | 좌장: 손호경 교수 (서울대학교) |
| 13:20~14:00 | Plenary Lecture | 좌장: 전준현 교수 (영남대학교) |
| | 이용환 교수 (서울대학교) | |
| 14:00~15:10 | S1. Human Health and Fungi | 좌장: 이경태 교수 (전북대학교) |
| | S1-1. 이승헌 박사 (연세대학교) | |
| | S1-2. 김주은 박사 (강원대학교) | |
| | S1-3. 조혜진 (경북대학교) | |
| | S1-4. 엄혜랑 (경상국립대학교) | |
| 15:10~15:30 | Break Time | |
| 15:30~16:45 | S2. Plant Pathogenic Fungi | 좌장: 정광우 박사 (한국원자력연구원) |
| | S2-1. 김경수 교수 (강원대학교) | |
| | S2-2. 김보민 (한국화학연구원) | |
| | S2-3. 신수빈 (서울대학교) | |
| | S2-4. 권구담 (서울대학교) | |
| 16:45~17:00 | Photo Time | |
| 17:00~18:00 | Dinner (스테이타워 Meal Time) | |
| 18:00~19:30 | S3. Yeast Genetics and Biotechnology | 좌장: 강현아 교수 (중앙대학교) |
| | S3-1. 김수린 교수 (경북대학교) | |
| | S3-2. 유수진 박사 (중앙대학교) | |
| | S3-3. 차승우 박사 (서울대학교) | |
| | S3-4. 김지원 박사 (KIST) | |
| 19:30~20:30 | Poster Presentation | |
| 2.2 (Fri) | 스테이타워 중연회장 SPACE B | |
| 09:00~12:00 | General Meeting & Group Discussion | |
| 12:00 | Closing Remark | |

Scientific Program

Plenary Lecture

PL

Plenary Lecture



13:20-14:00

The Rice Blast Fungus, *Magnaporthe oryzae*

Yong-Hwan Lee (Seoul National University)

Chair: Junhyun Jeon (Yeungnam University)

Symposium

S1

Human Health and Fungi

Chair: Kyung-Tae Lee (Jeonbuk National University)



S1-1 14:00-14:20

Identification of Essential Transcription Factors and Their Roles in *Cryptococcus neoformans*

Seung-Heon Lee (Yonsei University)



S1-2 14:20-14:40

Fine-tuning Histone Acetylation by Rpd31 and Rpd32 for Precision Control of *Candida albicans* Pathogenesis

Jueun Kim (Kangwon National University)



S1-3 14:40-14:55

The Function of a Myb-like Protein MylA in *Aspergillus flavus*

He-Jin Cho (Kyungpook National University)



S1-4 14:55-15:10

Gene Editing of *Ganoderma lucidum* by Cas9-gRNA Ribonucleoprotein Complex

Hyerang Eom (Gyeongsang National University)

S2

Plant Pathogenic Fungi

Chair: Kwang-Woo Jung (Korea Atomic Energy Research Institute)



S2-1 15:30-16:00

Deciphering the Biology of *Colletotrichum scovillei*, a Pepper Anthracnose Pathogen

Kyoung Su Kim (Kangwon National University)



S2-2 16:00-16:15

Edeine B₁ Produced by *Brevibacillus brevis* Reduces the Virulence of a Plant Pathogenic Fungus by Inhibiting Mitochondrial Respiration

Bomim Kim (Korea Research Institute of Chemical Technology)



S2-3 16:15-16:30

Oxaloacetate Anaplerosis Differently Contributes to Pathogenicity in Plant Pathogenic Fungi

Soobin Shin (Seoul National University)



S2-4 16:30-16:45

Identifying Transcription Factors of *Fusarium graminearum* Related with Fusarium graminearum Virus 2 Accumulation by Phenome-based Investigation

Gudam Kwon (Seoul National University)

S3

Yeast Genetics and Biotechnology

Chair: Hyun Ah Kang (Chung-Ang University)



S3-1 18:00-18:30

Yeast Metabolic Engineering for Sustainable Food and Bioprocess

Soo Rin Kim (Kyungpook National University)



S3-2 18:30-18:50

Exploration of Genomic and Functional Features of Non-conventional Yeast Species from Korean Traditional Fermented Foods

Su Jin Yoo (Chung-Ang University)



S3-3 18:50-19:10

Stereospecific (*S*)-Acetoin Production in *Saccharomyces cerevisiae*

Seungwoo Cha (Seoul National University)



S3-4 19:10-19:30

High-yield Lipid Production from Lignocellulosic Biomass Using Genetically Engineered *Yarrowia lipolytica*

Jiwon Kim (Korea Institute of Science and Technology)



Plenary Lecture

The 25th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2024

The Rice Blast Fungus, *Magnaporthe oryzae*

Yong-Hwan Lee

Department of Agricultural Biotechnology, Seoul National University


Rice blast is a compelling model system for studying host-parasite interactions due to its socio-economic impact and the availability of both the rice (2002) and fungal genomic sequences (2005). In an attempt to understand the molecular mechanisms of rice blast, Lee's group has been taking both forward and reverse genetics approaches. At the same time, his group has developed a bio-informatics portal system to archive enormous volumes of data on genetic diversities that exist among pathogen populations and genomes, which will be devoted to understand and predict the spatial and temporal variation of fungal pathogenesis.

Most significant contribution of Lee's group using reverse genetics approach was identifying and characterizing the genes involved in signal transduction pathways leading to appressorium formation in the rice blast fungus, *Magnaporthe oryzae*. This study was initiated by studying the roles of cAMP as a second messenger and later further deciphered by studying calcium- and MAP-kinase dependent pathways. A series of elegant works not only provided new insights on molecular mechanisms of the rice blast fungus but also directed new research areas in other plant pathogenic fungi. Another significant finding by Lee's group was systemically characterizing transcription factors genes in the genome of *M. oryzae*.

Using forward genetics strategies, Lee's group carried out a large-scale insertional mutagenesis of the *M. oryzae* strain KJ201 via *Agrobacterium tumefaciens*-mediated transformation, generating over 21,000 mutants. Using the novel functional genomics and informatics platform, Lee's group identified 201 new pathogenicity genes in rice blast fungus, the largest unbiased collection of pathogenicity genes ever discovered for a single species.

In addition to endeavors to decipher molecular understanding of the rice blast, Lee's group actively participated genome sequencing projects on both fungi and host plants. Lee's group also built a cyber-infrastructure as a bioinformatics portal system for analysis of such data in multiple contexts. The whole publicly available genome sequence information as well as most of the results from experimental biology is housed in customized and user-friendly databases.

In summary, Lee's group has been taking comprehensive and integrative approaches to understand the complex and subtle nature of fungal pathogenesis, using the technologies at the leading edge of the field. Understanding of fungal pathogenicity would provide not only new insights into the mechanisms and evolution involved in fungus-plant interactions but also the molecular basis on which efficient and stable crop protection strategies can be elaborated.



[S1] Human Health and Fungi

The 25th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2024

Identification of Essential Transcription Factors and Their Roles in *Cryptococcus neoformans*

**Seung-Heon Lee¹, Yu-Byeong Jang¹, Seong-Ryong Yu¹, Jin-Tae Choi¹,
Alexander Idnurm², Kyung-Tae Lee^{3*}, and Yong-Sun Bahn^{1*}**

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²School of BioSciences, The University of Melbourne, Parkville Campus, Parkville, VIC, 3010, Australia,

³Korea Zoonosis Research Institute, Jeonbuk National University

Cryptococcus neoformans causes cryptococcosis, which is one of the leading causes of death among HIV patients. Current antifungal treatments are limited, therefore, developing antifungal drugs is crucial. In this regard, essential proteins can be notable as they are required for growth and, therefore, considered as putative 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 17 TFs by replacing their promoters with the copper-regulated *CTR4* promoter. Under repressive condition, conditional expression strains of 10 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 performed spore analysis. After harvesting spores from the heterozygous mutant, we analyzed the genotypes of progenies and identified 16 TFs essential TFs of *C. neoformans*. To investigate the role of the essential TFs, we constructed constitutive overexpression strains and observed their phenotypic traits under various stress-containing conditions. Particularly, three TFs exhibited high divergence from other eukaryotes, and one of them showed significant involvement in antifungal drug susceptibility, stress responses, and the pathogenicity of *C. neoformans*. Our findings reveal the complicated signaling networks of the TF and indicate multiple essential TFs, giving the targets for cryptococcosis therapy.

Fine-tuning Histone Acetylation by Rpd31 and Rpd32 for Precision Control of *Candida albicans* Pathogenesis

Jueun Kim^{1,2} and Jung-Shin Lee^{1*}

¹Department of Molecular Bioscience, College of Biomedical Science, Kangwon National University,

²Kangwon Institute of Inclusive Technology, Kangwon National University

Rpd3 is a well-known Class I histone deacetylase found in yeast. *Candida albicans* possesses two distinct orthologous proteins, Rpd31 and Rpd32, yet the rationale behind their coexistence remains unclear despite previous studies focusing on their physiological roles. Additionally, the transcriptional regulatory mechanisms orchestrated by Rpd31 and Rpd32 through their catalytic functions remain elusive. In this study, we observed a mutual complementarity between Rpd31 and Rpd32 in orchestrating gene expression in *C. albicans*. Notably, their absence did not cause a global increase in histone acetylation but rather led to a redistribution of H3 acetylation from promoter-TSS regions into gene bodies. Genes that exhibited reduced expression in the absence of Rpd31/32 harbored extended upstream intergenic regions (IGRs) showing extensive H3 acetylation, which was depleted in the absence of Rpd31/32. Intriguingly, numerous transcription factors in *C. albicans* have long IGRs and their transcription were regulated by Rpd31/32. Consequently, the lack of Rpd31/32 resulted in impaired hyphal formation and complete loss of pathogenicity in mice. These findings demonstrate the essential role of Rpd31 and Rpd32 in regulating the precise positioning of H3 acetylation on various regulatory genes crucial for morphogenesis and virulence within host cells.

The Function of a Myb-like Protein MylA in *Aspergillus flavus*

He-Jin Cho¹, Ye-Eun Son¹, and Hee-Soo Park^{1,2*}

¹*School of Food Science and Biotechnology, Kyungpook National University,*

²*Department of Integrative Biology, Kyungpook National University*

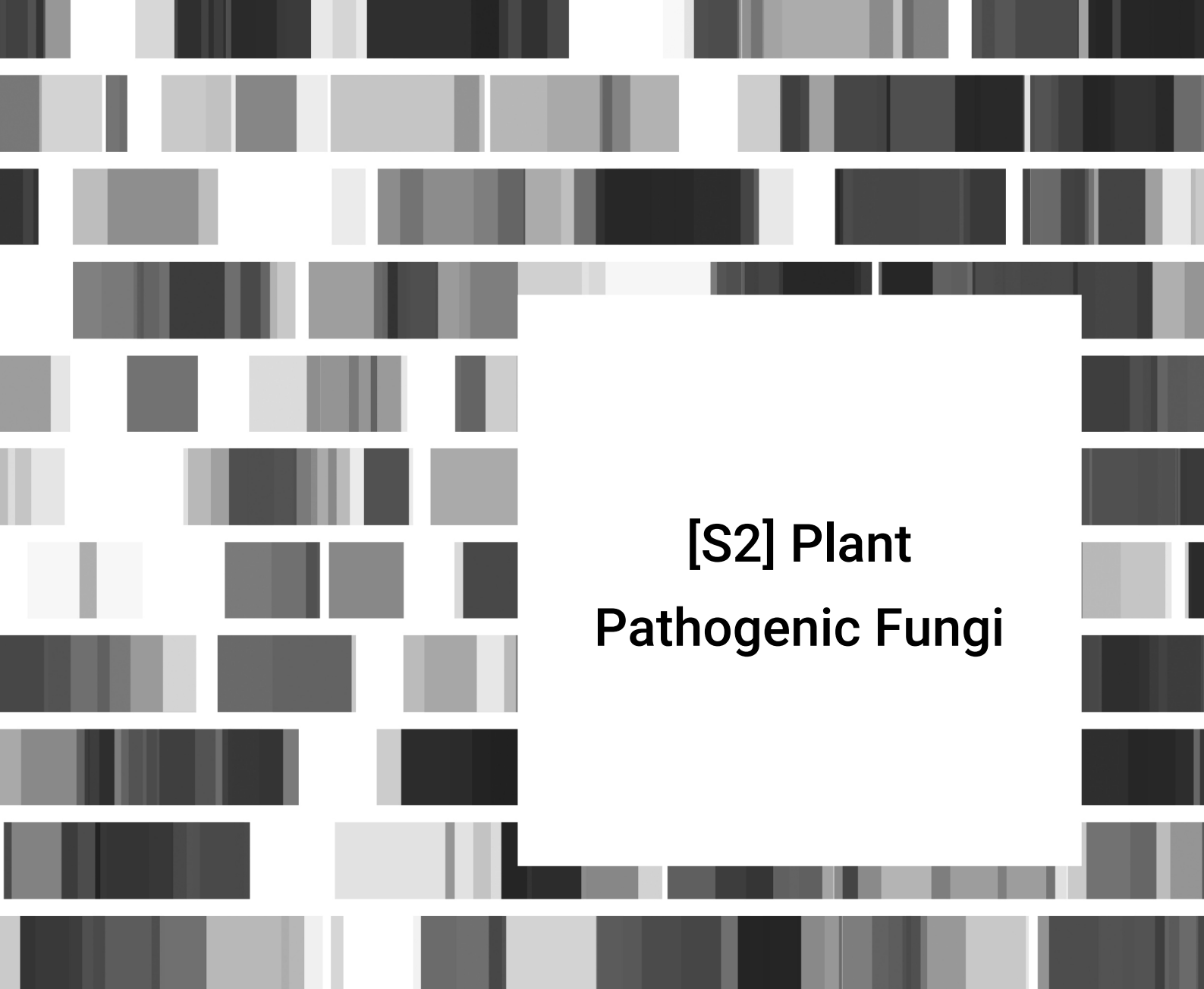
The representative filamentous fungi, *Aspergillus*, proliferate by producing conidia (asexual spores), and the procedures of conidia production (conidiation) are delicately regulated by a variety of transcription factors. Among them, Myb-like transcription factors (TFs) are one of the large TF family and modulate the levels of transcription or translation of their targets by sequence-specific DNA binding activities and protein-protein interaction activities. In our previous study, we identified 5 Myb-like (Myl) proteins that are highly expressed in the spore of *Aspergillus nidulans*. The deletion mutants (*mylA*[~]E) were generated and among them, the deletion of *mylA* showed impaired growth and conidiation in *A. nidulans* and *A. fumigatus*. To further characterize the conserved role of MylA among *Aspergillus* species, the *mylA* deletion mutant was generated in plant pathogenic fungus *Aspergillus flavus*, and phenotypic analysis was carried out. The *mylA*-deficient mutant showed reduced conidia production and colony growth compared to the control. Also, the *mylA* strain produced a reduced amount of sclerotia. Next, we analyzed the role of MylA in *A. flavus* conidia and found out deletion of *mylA* resulted in reduced trehalose content, spore viability, and stress tolerance in *A. flavus*. Lastly, we assessed the function of MylA in *A. flavus* pathogenicity by performing a kernel bioassay. When incubated with kernels, the *mylA* null mutant showed reduced conidia colonization and aflatoxin B1 production. Taken together, these results suggest that MylA plays pivotal roles in proper fungal development, conidial viability, and pathogenicity of *A. flavus*, and the functions of MylA in conidia are conserved in three *Aspergillus* species.

Gene Editing of *Ganoderma lucidum* by Cas9-gRNA Ribonucleoprotein Complex

Hyerang Eom and Hyeon-Su Ro*

*Department of Bio&Medical Bigdata (BK21plus) and Research Institute of Life Sciences,
Gyeongsang National University*

Gene editing of mushrooms has been performed using plasmid, but this causes foreign DNA insertion and therefore has a limitation of genetically modified organisms. In this research, the successful editing of *pyrG* gene in *Ganoderma lucidum* was accomplished using preassembled Cas9-gRNA ribonucleoprotein (RNP) complex. This complex primarily induced double-strand break (DSB) at the fourth base prior to the protospacer adjacent motif (PAM). Of the 66 transformants, 42 were deletion strains, and 30 had single deletions, and some of them resulted in large deletions involving promoter sequences. The remaining 24 transformants were inserted from 1 bp to 420 bp in size. These sequence originated from *G. lucidum* mitochondrial DNA (mtDNA) fragments, *E. coli* chromosomal DNA fragments, and the DNA of the Cas9 expression vector. Random integration of the DNA fragments upon the RNP-induced DSB event prompted us to investigate possible targeted insertion using a specific DNA fragment via non-homologous end joining (NHEJ), which was compared with the efficiency of the insertion through homologous recombination (HR). The result will be presented. This study demonstrated that gene editing of *G. lucidum* is achievable through the Cas9-gRNA complex with comparable efficiency to the plasmid-mediated editing system.



[S2] Plant Pathogenic Fungi

The 25th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2024

Deciphering the Biology of *Colletotrichum scovillei*, a Pepper Anthracnose Pathogen

Teng Fu, Yong-Won Song, Hui-Ju Han, and Kyoung Su Kim*

*Division of Bio-Resource Sciences, Interdisciplinary Program in Smart Agriculture, and Agriculture
and Life Sciences Research Institute, Kangwon National University*

Anthracnose is a cosmopolitan disease of many plants, caused by ascomycete *Colletotrichum* species. The disease has long been a serious threat in agriculture, especially on many fruits worldwide. Different species of *Colletotrichum* are known to cause anthracnoses on one plant and others, which would be a substantial obstacle in development of resistant cultivars as well as disease management. Therefore, understanding molecular biology underlying the polycyclic dissemination and plant infection of *Colletotrichum* species is a prerequisite for developing a novel strategy for anthracnose control. We have been working over the last decade on *C. scovillei*, infecting economically important fruits and being a dominant species on peppers in many countries. Infected pepper fruits typically exhibit sunken necrotic spots of anthracnose within a few days, leading to a considerable damage in the production. Our lab attempts to delineate gene and protein functions and interactions from “-omics” screening and projects through functional genomics to advance basic knowledge and its translation into application to benefit agriculture and other sectors. The current state of our research includes how *C. scovillei* progresses to a stage of infection, compromising host defenses and dissemination. We hope that our presentation would guide the development of key baseline and help move forward for research across diverse microbial taxa and environments.

Edeine B₁ Produced by *Brevibacillus brevis* Reduces the Virulence of a Plant Pathogenic Fungus by Inhibiting Mitochondrial Respiration

Bomin Kim^{1,2}, Minh Van Nguyen^{1,2}, Yeong Seok Kim^{1,2}, Jae Woo Han¹, Joo-Youn Lee³, Junhyun Jeon⁴, Gyung Ja Choi^{1,2}, and Hun Kim^{1,2*}

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²Department of Medicinal Chemistry and Pharmacology, University of Science and Technology,

³Therapeutics and Biotechnology Division, Korea Research Institute of Chemical Technology,

⁴Department of Biotechnology, Yeungnam University

Plant pathogenic fungi cause serious diseases, which result in the loss of crop yields and reduce the quality of crops worldwide. To counteract the escalating risks of chemical fungicides, interest in biological control agents to manage plant diseases has significantly increased. In this study, we comprehensively screened microbial culture filtrates using a yeast screening system to find microbes exhibiting a respiratory inhibition activity. Consequently, we found a soil-borne microbe *Brevibacillus brevis* HK544 strain exhibiting a respiration inhibitory activity and identified edeine B₁ (EB₁) from the culture filtrate of HK544 as the active compound of the respiration inhibition activity. Furthermore, against a plant pathogenic fungus *Fusarium graminearum*, our results showed that EB₁ has effects on multiple aspects of respiration with the down-regulation of most of the mitochondrial-related genes based on transcriptome analysis, differential EB₁-sensitivity from targeted mutagenesis, and the synergistic effects of EB₁ with electron transport chain complex inhibitors. With the promising plant disease control efficacy of *B. brevis* HK544 producing EB₁, our results suggest that *B. brevis* HK544 has potential as a biocontrol agent for Fusarium head blight.

Oxaloacetate Anaplerosis Differently Contributes to Pathogenicity in Plant Pathogenic Fungi

**Soobin Shin^{1†}, Seong-Hun Bong^{1†}, Heeji Moon¹, Hosung Jeon¹, Jung-Eun Kim³,
Hun Kim², Gyung Ja Choi², Do Yup Lee^{1,3*}, and Hokyoung Son^{1,3*}**

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Anaplerosis refers to enzymatic reactions or pathways replenishing metabolic intermediates in the tricarboxylic acid (TCA) cycle. Pyruvate carboxylase (PC) plays an important anaplerotic role by catalyzing pyruvate carboxylation, forming oxaloacetate. Although PC orthologs are well conserved in prokaryotes and eukaryotes, their pathobiological functions in filamentous pathogenic fungi have yet to be fully understood. Here, we delve into the molecular functions of PC in *Fusarium graminearum* and *F. oxysporum*, prominent fungal plant pathogens with distinct pathosystems, demonstrating variations in carbon metabolism for pathogenesis. Surprisingly, the PC deletion mutant of *F. oxysporum* exhibited pleiotropic defects in hyphal growth, conidiation, and virulence, unlike *F. graminearum*, where PC mutation did not significantly impact virulence. To further explore the species-specific effects of PC deletion on pathogenicity, we conducted comprehensive metabolic profiling. Despite shared metabolic changes, distinct reprogramming in central carbon and nitrogen metabolism was identified. Specifically, alpha-ketoglutarate, a key link between the TCA cycle and amino acid metabolism, showed significant down-regulation exclusively in the PC deletion mutant of *F. oxysporum*. The metabolic response associated with pathogenicity was notably characterized by S-methyl-5-thioadenosine and S-adenosyl-L-methionine, known for their crucial role in plant pathogenicity. This research shed light on how PC-mediated anaplerosis affects fungal metabolism and reveals species-specific variations, exemplified in *F. graminearum* and *F. oxysporum*.

Identifying Transcription Factors of *Fusarium graminearum* Related with *Fusarium graminearum* Virus 2 Accumulation by Phenome-based Investigation


Gudam Kwon¹, Jisuk Yu^{2*}, and Kook-Hyung Kim^{1,2,3*}

¹Department of Agricultural Biotechnology, Seoul National University

²Plant Genomics and Breeding Institute, Seoul National University

³Research Institute of Agriculture and Life Sciences, Seoul National University

The *Fusarium graminearum* virus 2 (FgV2) is a mycovirus infecting *Fusarium graminearum*. FgV2 has 5 segmented dsRNA genome and FgV2 infection to *F. graminearum* causes a hypovirulence entailing reduced mycelial growth and an alteration on transcriptome. To identify TFs that might be related to FgV2 infection, we transferred FgV2 to the TF gene deletion mutant library of *F. graminearum*. When we measured mycelial growth and FgV2 accumulation level in several mutants, we observed a tendency that the FgV2 accumulation level was high as the mycelial growth rate reduced. We proceeded further study focusing on the TFs related to DNA damage response, oxidative stress response, and RNA interference. Among the TF mutants related to DNA damage response, Δ GzGATA007 mutant which is resistant to hydroxyurea (HU) became sensitive to HU upon FgV2 infection. In addition, FgV2 accumulation level in Δ GzGATA007 was increased in colonies grown on HU containing media. With oxidative stress response related TFs, we observed that ROS accumulation was dramatically changed upon FgV2 infection in Δ GzWING020 and Δ GzZC121 which are associated with oxidative stress response. By semi-quantitative PCR, it was revealed that some ROS-generating and -scavenging gene expression was altered after FgV2 infection in those mutants. Using RT-qPCR with FgV2-infected TF mutants, we can find several putative TFs which might be involved in the transcriptional regulation of *FgDICER-2* and *FgAGO-1*, which are responsible for RNAi pathway against mycovirus infection in *F. graminearum*. Among them, we have conducted further study with 2 genes which are expected to work on post-transcriptional or post-translational regulation process. Taken together, through FgV2 transmission into *F. graminearum* TF deletion library, we investigated TFs which affect FgV2-infected colony morphology and various cellular processes which seem to be related to FgV2 accumulation in the host.



[S3] Yeast Genetics and Biotechnology

The 25th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2024

Yeast Metabolic Engineering for Sustainable Food and Bioprocess

Soo Rin Kim

School of Food Science and Biotechnology, Kyungpook National University

Industrial biotechnology based on yeast fermentation is a promising strategy that can alleviate global warming and climate change. However, *Saccharomyces cerevisiae*, widely used in bioprocesses, releases a large amount of carbon dioxide (CO₂) during fermentation. This study developed a mixotrophic CO₂-fixing *S. cerevisiae* to achieve carbon neutrality and sustainability in bioprocess. A CO₂-fixation pathway was constructed in a xylose-utilizing *S. cerevisiae* by heterologous expression of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and phosphoribulokinase (PRK). Furthermore, a delta-integration strategy was utilized, and the RuBisCO gene copy number was increased to 10 copies to improve the efficiency of CO₂-fixation. An additional Cas9-based genome editing was performed to overexpress other CO₂-fixation related genes. The resulting CO₂-fixing yeast, SJ03, exhibited the highest RuBisCO activity. During anaerobic xylose fermentation, ethanol concentration was increased by 17% and ethanol yield was increased by 16% compared to the control strain. In addition, CO₂ emissions decreased by 7%. These results suggest that overexpression of the CO₂-fixation pathway coupled with xylose utilization in *S. cerevisiae* might reduce CO₂ emission in bioprocesses.

Exploration of Genomic and Functional Features of Non-conventional Yeast Species from Korean Traditional Fermented Foods

Su Jin Yoo, Da Min Jeong, Hyeon Jin Kim, and Hyun Ah Kang*

Department of Life Science, Chung-Ang University

In Korean traditional fermented foods, diverse yeast species other than *S. cerevisiae*, so-called non-conventional yeasts, participate in fermentation with defined and as yet-unidentified functions. In this study, we introduce the genomic and physiological features of several yeast species mainly isolated from Nuruk, a starter for traditional Korean rice wines, and Jang, a traditional Korean fermented soy product. A variety of yeast species have been isolated from Nuruk and the Jang products, including *Saccharomycopsis fibuligera*, *Hyphopichia burtonii*, *Debaryomyces hansenii*, which belong to the CUG clade showing high osmotic tolerance, and *Wickerhamomyces subpelliculosus*, which is little known, but has been studied as an alternative baker's yeast. Comparative genomics revealed that the interspecies hybridization within yeast species occurs frequently for generating heterozygous diploid genomes as an evolutionary strategy in the fermentation environment of Nuruk and Jang. Through the omics analyses, novel genes involved in cellulose degradation and volatile aroma biosynthesis useful for biotechnological application have been discovered in *S. fibuligera*, while for *Hyphopichia* yeasts we gained insights into the novel mechanisms of halo- and osmo-tolerance for survival in fermentation environments. Interestingly, one of the Jang yeast isolates, *D. hansenii*, showed probiotic potential with survival in the presence of bile salts and at low pH, and the immune-modulating activity to induce high level of an anti-inflammatory cytokine, IL-10. Another Jang yeast isolate, *W. subpelliculosus*, produced high level of ethyl acetate comparable to that from *Wickerhamomyces anomalus*, a major ethyl acetate producer. *In silico* analysis validated by functional analysis led to the identification of novel *W. subpelliculosus* genes involved in formation of volatile aroma esters, useful for developing microbial cell factories for volatile flavor production and supportive of the yeast as a new key player in food industry. Altogether, this study based on the high-quality whole-genome information with systematic characterization of physiological features of yeast species will contribute to improving and globalizing Korean traditional fermented products with high quality and functionalities.

Stereospecific (*S*)-Acetoin Production in *Saccharomyces cerevisiae*

**Seungwoo Cha¹, Byeongseon Jang², Daeyeol Lee², InJae Cho², Youngmin Lee²,
Hyesoo Shin², and Ji-Sook Hahn^{2*}**

¹Bio-MAX/N-Bio, Institute of BioEngineering, Seoul National University,

²School of Chemical and Biological Engineering, Seoul National University

Acetoin, a four-carbon compound containing one chiral center, is used as a buttery flavoring ingredient used in the food and cosmetics industries. It is important to isolate the stereoisomers of acetoin ((*R*)-acetoin and (*S*)-acetoin) for pharmaceutical applications or stereoselective synthesis, but due to the complex chemical process for purification of each acetoin forms, biosynthetic approaches have gained a growing interest. Several researches have already succeeded in producing (*R*)-acetoin with both high yield and purity.

However, prior studies have mainly focused on the conversion of glucose into (*R*)-acetoin in various microorganisms, while (*S*)-acetoin has predominantly been produced through direct enzymatic conversion of diacetyl or meso-2,3-butanediol. Within the metabolic pathway of acetoin, α -acetolactate is a crucial intermediate that is toxic to cell. While (*R*)-acetoin can be rapidly converted from α -acetolactate through enzymatic reactions, (*S*)-acetoin should undergo spontaneous decarboxylation under aerobic condition. This non-enzymatic reaction is a major bottleneck to produce (*S*)-acetoin in microbial system. In fact, there has been only one study that has attempted to produce (*S*)-acetoin from glucose in *Lactococcus lactis*.

In this study, we introduced three strategies within *Saccharomyces cerevisiae* to facilitate the fermentative production of (*S*)-acetoin from glucose: (1) Elimination of the endogenous acetoin biosynthesis pathway, (2) Enhancement of the spontaneous reaction that converts α -acetolactate to diacetyl, and (3) Enzyme engineering of α -acetolactate decarboxylase to achieve reversed stereoselectivity. These strategies successfully enhanced the stereospecificity and productivity of (*S*)-acetoin in *S. cerevisiae*.

High-yield Lipid Production from Lignocellulosic Biomass Using Genetically Engineered *Yarrowia lipolytica*

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The escalating concerns regarding climate change and global warming have led to the exploration of alternative renewable energy sources. The non-conventional yeast, *Yarrowia lipolytica*, emerges as a promising platform for lignocellulosic biodiesel and bio-jet fuel production owing to its outstanding lipid accumulation capacity. However, the main obstacle to using lignocellulosic biomass in *Y. lipolytica* is its inability to utilize xylose, the second most abundant sugar in lignocellulosic biomass. To this end, we have developed an isomerase-based xylose utilizing strain of *Y. lipolytica*. The construction of this strain involved the application of adaptive laboratory evolution, from which critical mutations were revealed through genotypic analysis. Among the 532 mutations identified, the deletion of yIXR1 (YALI0D07634g) emerged as a crucial factor enabling isomerase-based xylose utilization in *Y. lipolytica*. Based on the insights from evolved strains, a genetically engineered strain of YWX was developed, resulting in a 5.7-fold increase in lipid production compared to the wild-type strain during glucose/xylose co-fermentation. Overall, our findings contribute to enhancing the capability of *Y. lipolytica* in lipid production using a carbon-neutral feedstock of lignocellulosic biomass.



Poster

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Uncovering the Roles and Regulatory Mechanism of the TOR-Ypk1-Fpk1 Signaling Pathway in *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungus, primarily causing meningitis in immunocompromised individuals. A crucial mechanism for cellular homeostasis and signaling in this fungus involves the transport of phosphatidylserine and phosphatidylethanolamine by flippase from the plasma membrane's outer leaflet to the inner leaflet. Our study investigates the regulatory function of the TOR-Ypk1-Fpk1 signaling pathway on flippase activity in *C. neoformans*. The antibiotic duramycin, a phosphatidylserine binder, served as our test agent. Our results indicate that Ypk1 acts as a positive regulator for Fpk1, as evidenced by the restoration of duramycin resistance through *FPK1* overexpression in *ypk1Δ*. This trend was also observed when exposed to osmotic stressors like NaCl and membrane stressors like SDS. Phagocytic killing tests showed an increased vulnerability in *ypk1Δ* compared to the wild type, while *FPK1* overexpression in *ypk1Δ* negated this susceptibility. However, the similarity between the phagocytic killing of *fpk1Δ* and the wild type suggests additional regulatory factors for the flippase pathway beyond Fpk1. Our research also indicates the functional role of Ypk101, a paralog of Ypk1, in restoring partial growth under osmotic stress (1 M NaCl and KCl) conditions when overexpressed in *ypk1Δ*. The aim of this study is to explore the TOR-Ypk1-Fpk1 pathway, presumed to regulate the flippase complex in *C. neoformans*, and to elucidate the interactions between these components.

Unveiling the WD40 Repeat-containing Protein Networks Regulating the Pathogenicity of *Cryptococcus neoformans*

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Protein-protein interaction (PPI) is crucial for biological functionality, with the WD40 domain playing a pivotal role. Yet, the function of WD40 proteins in the pathogenicity of human fungal pathogens is still unclear. Our study scrutinised the roles of canonical WD40 proteins in *Cryptococcus neoformans*, a global fungal pathogen causing fatal meningoencephalitis. We identified 94 canonical WD40 proteins in the *C. neoformans* genome and constructed 103 signature-tagged gene deletion strains for 52 WD40 proteins. Our *in vitro* and *in vivo* analyses revealed WD40 proteins significantly contributing to virulence and characterized their interaction networks. Furthermore, the potential essentiality of 37 WD40 proteins was verified through conditional inhibition or sporulation analysis. Our finding enriches the understanding of WD40 protein-dependent PPI networks in fungal pathogenicity and offers insight into potential antifungal drug development through PPI inhibitors.

Elucidating the Cryptic Functions of Mitogen-activated Protein Kinases Cpk2 and Mpk2 in *Cryptococcus neoformans*

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In basidiomycetous human fungal pathogen, *Cryptococcus neoformans*, has five mitogen-activated protein kinases; Mpk1, Cpk1, and Hog1 play central roles in various physiological functions. Apart from these three major MAPKs, *C. neoformans* has Cpk2 and Mpk2, which are paralogs of Cpk1 and Mpk1, respectively, but their roles remain elusive. Our previous genome-wide functional analysis of cryptococcal kinases revealed that Cpk2 plays minor roles in osmotic and genotoxic stress response and melanin production but is dispensable for the mating process, unlike Cpk1. Deletion of *CPK2* does not lead to defects in virulence and infectivity of *C. neoformans*. Similarly, unlike Mpk1, Mpk2 plays minor roles in cell membrane stress response, resistance to fludioxonil and fluconazole, and melanin as well as urease production. In this study, we aimed to elucidate the functional connection of Cpk2 and Mpk2 to Cpk1- and Mpk1-dependent signaling pathways in *C. neoformans*. In support of their phylogenetic relationship, here we provide the following experimental evidence showing that Cpk2 and Mpk2 play redundant roles with Cpk1 and Mpk1, respectively. Overexpression of *CPK2* could restore the mating defect of *cpk1Δ*, including mating pheromone production, filamentation, and sporulation. Then, overexpression of *MPK2* could also partially restore the growth defect of *mpk1Δ* under cell wall destabilizing conditions as well as restore the basal urease production level.

[This research was supported by NRF.]

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

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Phosphatases play critical roles in regulating cellular networks involved in the survival and virulence of fungal pathogens. Specifically, protein phosphatase 2A (PP2A) is a highly conserved serine-threonine phosphatase composed of catalytic, scaffold, and regulatory subunits. In this study, we aim to unravel the signaling networks of a PP2A-like phosphatase *SIT4* in *Cryptococcus neoformans*, an opportunistic fungal pathogen. From our previous study, we have identified *SIT4* as a virulence-related phosphatase that promotes blood-brain barrier adhesion and crossing. To elucidate the signaling pathway 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. Both *sit4Δ* and *sap190Δ* displayed increased susceptibility against rapamycin, and *sap190Δ* showed reduced BBB crossing but at a reduced severity compared to *sit4Δ*. Also, because the TOR (target of rapamycin) pathway regulates cell growth and metabolism, 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, and in basal condition, *SIT4* transcription increased in *sap190Δ* while *SAP190* transcription increased in *sit4Δ*. From here, we aim to identify the signaling networks of *SIT4* to uncover its role and mechanism in brain infection.

[This research was supported by NRF.]

Unveiling Regulatory Mechanism of the HAMP-domain-containing Hybrid Histidine Kinase Tco1 in the Phosphorelay System of Human Fungal Pathogen *Cryptococcus neoformans*

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The multicomponent phosphorelay system plays a critical role in fungal pathogenesis by controlling virulence factor production and developmental processes. In this study, the group III HHKs, which are most widely conserved in fungi among HHKs, was analyzed in *Cryptococcus neoformans*, a fungal meningoencephalitis pathogen. Individual and sequential domain deletion studies indicated that each N-terminal extension, seven HAMP, and histidine kinase-receiver (HK-Rec) domain was required for the whole Tco1 functions. The penultimate and last HAMP domains play distinct roles in the autoregulation of Tco1, which ultimately affects the Hog1 MAPK. Bimolecular fluorescence complementation assays demonstrated that Tco1 weakly interacted with histidine-containing phosphotransfer protein (HPt) Ypd1 under basal conditions but more strongly upon fludioxonil treatment. This led to the subsequent interaction of Ypd1 with cytosolic response regulator (RR) Ssk1 and nuclear RR Skn7. The yeast two-hybrid assays demonstrated that the interaction between HAMP and HK-Rec or HAMP domains could dimerize Tco1. Overall, this study characterizes the function of each domain and regulatory mechanisms of Tco1 in association with the multicomponent phosphorelay system and the Hog1 MAPK pathway.

Blocking the Hydroxylation of Sphingolipid in *Yarrowia lipolytica* for Production of Human-type Long Chain Bases and Glucosylceramides

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Sphingolipids serve as vital membrane components in mammalian cells, plants, and various microbes. We characterized and engineered the sphingolipid biosynthesis pathway in an oleaginous and dimorphic yeast *Yarrowia lipolytica*. To block the fungal-specific phytosphingosine (PHS) pathway, the *SUR2* gene, encoding a sphinganine C4-hydroxylase, was disrupted, which resulted in remarkably elevated secretory production of dihydrosphingosine (DHS) and sphingosine (So), along with increased glucosylceramides (GlcCers) production. The *Ylsur2Δ* displayed a retarded growth with increased pseudohyphal formation, stress sensitivity, and distinct changes of inositolphosphorylceramides (IPCs) and sterols. RNA Seq-based transcriptome analysis indicates that the altered profiles of long chain bases (LCBs) and GlcCers observed in *Ylsur2Δ* are considerably consistent with the altered expression of the genes in sphingolipid pathway of *Y. lipolytica*. Subsequently disrupting the *SLD1* gene, encoding the fungal-specific Δ8 sphingolipid desaturase, restored filamentous growth of *Ylsur2Δ* to the yeast-type form and enhanced the production of human-type GlcCers. Introducing mouse ceramidase 1 into the *Ylsur2Δsld1Δ* mutants significantly escalated DHS and So production. Engineered *Y. lipolytica* shows high potential for producing non-acetylated LCBs and human-type GlcCers, valuable ingredients for in pharmaceuticals, cosmeceuticals, and nutraceuticals.

Unraveling the Evolutionary Unique Glycoprotein Quality Control System and Its Roles in Cellular Fitness and Extracellular Vesicle Transport in *Cryptococcus neoformans*

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To ensure the accurate folding of glycoproteins, eukaryotes evolved a highly conserved *N*-glycan-dependent endoplasmic reticulum protein quality control (ERQC) system. In this study, we investigated the unique features and functions of the ERQC system in the human pathogen *Cryptococcus neoformans*, by analyzing the *uggt* Δ mutant lacking UDP-glucose: glycoprotein glucosyltransferase, and the *mns1A* Δ *1B* Δ double mutant lacking α 1,2-mannosidases, involved in trimming mannose residues from the *N*-oligosaccharides. All mutants consistently showed alterations in the *N*-glycan profiles, defective capsule formation, and defective cell surface organization. Remarkably, *uggt* Δ exhibited increased sensitivity to various stresses and severe impairment particularly in extracellular vesicle-mediated secretion of virulence factors, leading to a substantial decrease in survival inside host cells and consequent loss of virulence *in vivo*. Comparative transcriptome analysis uncovered increased ER stress in *uggt* Δ , evidenced by the upregulation of genes involved in protein folding, proteolysis, and cell wall remodeling, whereas there were no observable differences in the expression of genes involved in protein secretion or capsule biosynthesis. In summary, our findings emphasize the essential role of the *N*-glycan-dependent ERQC system in cellular fitness under adverse conditions, such as the host environment, and in the extracellular transport of virulence factors, which are crucial for full fungal pathogenicity.

***S. cerevisiae* Engineering for Production of Ceramide and Shingoid Bases**

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Unlike most higher eukaryotes and other yeast species, *S. cerevisiae* lacks the *DES1* gene, encoding delta 4-desaturase, thus producing only phytosphingosine(PHS)-containing sphingolipids, mainly such as inositolphosphorylceramide (IPC), without production of glycosylceramides. Here, we first engineered *S. cerevisiae* by introducing the *Pichia ciferrii SLI1* gene, encoding N/O-acetyltransferase associated with long chain bases (LCBs) acetylation, and additionally overexpressing the *S. cerevisiae* ceramidase genes (*ScYPC1*, *ScYDC1*), which resulted in remarkable secretory production of acetylated LCBs pools including 3Ac-phytosphingosine, 2Ac-dehydrosphingosine as dominant products. Specifically, the WT/PcSLI1/ScYPC1 strain with the introduction of Ypc1p ceramidase exhibited the highest production of secreted LCBs. Another strategy for human type ceramide overproduction involved the introduction of a set of human and *F. graminearum DES1* genes into the *S. cerevisiae* triple null mutant (*Scypc1Dydc1Dsur2D*). The deletion of ceramidase genes was aimed to block the degradation of ceramide and the deletion of *SUR2*, encoding a sphingolipid $\Delta 4$ -desaturase, was made to block PHS-based ceramides. Unexpectedly, instead of production of sphingosine-containing ceramide, an increased production of 3-ketodehydrosphingosine, the initial product in sphingosine biosynthesis, was observed the *Scypc1Dydc1Dsur2D/DES1* strain. Our results present the potential of engineered *S. cerevisiae* strains to increase the secretory production of sphingoid bases with high industrial potential as pharmaceutical and cosmeceutical ingredients.

Efficient Production of Naringenin and Phloretin in *Saccharomyces cerevisiae* via Metabolic Engineering

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Flavonoids, commonly found in plants, exhibit diverse biological activities such as antioxidant and anti-inflammatory properties. Consequently, they find applications in various industries including pharmaceuticals, food, supplements, and cosmetics. Current production of these flavonoids relies on plant extracts, limited by spatial and temporal constraints in cultivation. Producing them in microorganisms can overcome these limitations and ensure consistent quality and quantity. Naringenin serves as a central compound for synthesizing other flavonoids, including quercetin, catechin, and anthocyanidin. To produce naringenin in *Saccharomyces cerevisiae*, four heterologous genes-tyrosine ammonia lyase (TAL), 4-coumarate: coenzyme ligase (4CL), chalcone synthase (CHS), and chalcone isomerase (CHI)-are necessary. Phloretin, a dihydrogen chalcone-class flavonoid abundant in apples and strawberries, acts as the precursor to trilobatin, a plant-derived sweetener. This is a byproduct observed during the production of naringenin. In this study, we achieved efficient production of naringenin and phloretin in *S. cerevisiae* by enhancing the flux towards the target compounds through the elimination and down regulation of competing pathways, the introduction of mutations to confer resistance of feedback inhibition, and engineering of enzymes for the production of target molecules. These strains can be utilized as platform strains for the production of diverse flavonoids and natural sweetener.

Efficient Production of Retinoic Acid Based on Biosensor in *Saccharomyces cerevisiae*

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Retinoic acid (RA), a derivative of vitamin A comprising four isoprene units arranged in a head-to-tail structure, is a widely used medication approved by the FDA, primarily for treating acne and various skin conditions due to its diverse physiological functions. Despite the growing demand for RA in the pharmaceutical industry, the biosynthesis process of RA from microorganisms is still in its early stages, particularly when compared to other retinoids like retinol and retinal. The challenge lies in identifying efficient retinal dehydrogenases crucial for RA production from retinal. Since RA biosynthesis pathway is complicated and dynamic, it is difficult to identify and control the genes modulating RA, and its precursors. To overcome these obstacles, we have introduced an RA-biosensor as an *in vivo* gene screening system from large-scale libraries. Retinoic acid serves as a ligand for retinoid nuclear receptors such as retinoid X receptor (RXR) and retinoic acid receptor (RAR). It activates the RXR-RAR dimer with coactivators, forming a transcription factor for the target genes. These protein complexes bind to specific retinoic acid response elements (RAREs) located in the target gene promoters, thereby regulating their expression. Utilizing this mechanism, RA-biosensor was developed in *Saccharomyces cerevisiae*, where the reporter genes are expressed under the control of the RAREs. We also generated an RA-production *S. cerevisiae* strain by introducing biosynthetic genes and strengthening the metabolic pathways. The RA-biosensor will be introduced into the RA-producing strain to screen improved RA-production strains via adaptive laboratory evolution (ALE).

Engineering α -Acetolactate Decarboxylases to Shift Stereoselectivity from (*R*)- to (*S*)-Acetoin

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Acetoin, a key bulk chemical with diverse applications such as the synthesis of α -hydroxyketone derivatives, exists in (*R*) and (*S*) stereoisomers. Establishing an environmentally friendly and safe platform for the selective production of (*R*)/(*S*)-acetoin is crucial for its versatile use in chemical syntheses. Current microbial fermentation processes, catalyzed by α -acetolactate decarboxylase (ALDC), exclusively yield (*R*)-acetoin, rendering the biosynthesis of (*S*)-acetoin unattainable. In this study, we successfully altered the stereoselectivity of *Bacillus subtilis*-derived ALDC (*Bs*ALDC), expressed in *Saccharomyces cerevisiae*, creating a fungal platform for (*S*)-acetoin biosynthesis. Comparative analysis with *Streptococcus thermophilus* ALDC (*St*ALDC) identified key residues influencing stereoselectivity based on the number of carbon atoms in substrates. Through strategic mutations, we improved stereoselectivity towards (*S*)-acetoin to 86.9%. Further investigations identified residues with a *pKa* near 5 in the side chain, enabling activity only at specific pH. A mutation in this residue in the mutant increased activity, resulting in a higher (*S*)-acetoin titer of 655 mg/L. This study marks the first microbial fermentation for the production of both acetoin isomers, achieved through ALDC engineering. The developed fungal-based platform demonstrates promise for environmentally conscious acetoin production for various chemical applications.

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

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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, generally showed similar phenotypes as *cna1Δ* and *cnb1Δ* but at reduced severity. To test whether Crz1 is the downstream factor of calcineurin, we examined the localization of Crz1 using a fluorescent tag. We identified that Crz1 was localized inside the nucleus in response to high temperature. We also discovered that *crz2Δ* did not display the phenotypes related to the 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*.

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Proteomic Analysis of Cell Wall Proteins with Various Linkages in *Fusarium graminearum*

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The fungal cell wall, primarily comprising a glucan-chitin matrix and cell wall proteins (CWPs), serves as a key mediator for fungal interactions with the environment and plays a pivotal role in virulence. In this study, we employed a comprehensive proteomics approach to analyze the CWPs in the plant pathogenic fungus, *Fusarium graminearum*. Our methodology successfully extracted and identified 1,373 CWPs, highlighting their complex linkages, including non-covalent bonds, disulfide bridges, alkali-sensitive linkages, and glycosylphosphatidylinositol (GPI) anchors. A significant subset of these proteins, enriched in Gene Ontology terms suggests multifunctional roles of CWPs. Through the integration of transcriptomic and proteomic data, we observed differential expression patterns of CWPs across developmental stages. Specifically, we focused on two proteins, Fca7 and Cpd1, which were upregulated *in planta* and confirmed their localization predominantly outside the plasma membrane, primarily in the cell wall and periplasmic space. The disruption of *FCA7* reduced virulence on wheat, aligning with previous findings, and underscoring its significance. Overall, our findings offer a comprehensive proteomic profile of CWPs in *F. graminearum*, laying the groundwork for a deeper understanding of their roles in development and interactions with host plants.

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Novel Nuclear Localization Sequence of MoHTR1, a Nuclear Effector of the Rice Blast Fungus, is Crucial for Fungal Pathogenicity and Host Genes Transcriptional Reprogramming

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Plant pathogen's effectors are secreted into the host and play pivotal roles in modulating the host immune system. Nuclear effectors are translocated into host nuclei and interact with proteins and DNA to regulate various biological processes. Nuclear localization sequence (NLS) is the most well-known factor for facilitating the protein translocation. However, the molecular mechanism of NLS-associated transport vehicles and the roles of NLS in the transcriptional reprogramming of pathogenic effectors remain enigmatic. Our previous study reported on MoHTR1, a nuclear effector of the rice blast fungus, which is translocated to rice nuclei but not fungal nuclei. In this study, we identified the core sequence (RxKK) of NLS responsible for MoHTR1's nuclear localization. MoHTR1 was localized in the host nucleus through interaction with rice importin α . MoHTR1 NLS facilitates its escort the cytoplasmic effectors of *Magnaporthe oryzae* into rice nuclei. Furthermore, nuclear effector candidates and rice proteins that have the RxKK sequence also exhibited nuclear localization, highlighting the crucial role of RxKK sequence in this process. Additionally, we unveiled the importance of SUMOylation, post-translational modification, in the secretion and translocation of MoHTR1 to biotrophic interfacial complexes and host nuclei. Moreover, MoHTR1 NLS was essential for the pathogenicity of *M. oryzae* by reprogramming immunity-related genes in the host. Our findings will provide unprecedented insights into the significance of NLS on nuclear effector and its role in pathogen-host interactions.

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Biological Insight into the Ectomycorrhizal Symbiosis of *Tricholoma matsutake* Using Shotgun Metagenomic Sequencing

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It is widely recognized that plant species establish symbiotic relationships with microbial partners in soil to overcome nutrient limitations. In plant-fungus symbiosis, it is well known that mycorrhizal partners provide inorganic phosphate and nitrogen and take carbohydrates in various forms as a reward. However, the proteins or genes that have functions with the translocation of nutrients remain ambiguous. To address this unclear part, we collected soil samples from the *Tricholoma matsutake* habitat and performed metagenomic shotgun sequencing to investigate functional expressions during the symbiotic interaction between the ectomycorrhizal (ECM) fungus and its host plant, *Pinus densiflora*. To identify the key functions of ECM fungus, we divided the habitat into two areas, one where mycorrhizal symbiosis was active, and another where it was not. The active area contained the Shiro, which is the mycelial aggregate of *T. matsutake*, in the soil, while the inactive area did not have any visible signs of *T. matsutake*. Therefore, functional profiling in these two areas is assumed to give novel biological insights into ECM interactions.

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Uncovering the Roles of the Snf1 Kinase Complex in the Nutrient Sensing, Stress Responses, and Pathogenicity of *Cryptococcus neoformans*

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Cryptococcus neoformans, an opportunistic human fungal pathogen, induces deadly meningoencephalitis, with its ability to cross the blood-brain barrier (BBB) significantly influenced by fungal kinases. Our research, built on a kinase mutant library and *in vivo* transcriptomes analysis, identified 18 key kinases regulating BBB traversal and virulence. In this study, we particularly focus on the Snf1 kinase, a key player in the carbon source sensing pathway in *C. neoformans*, consisting of three subunits: α -subunit Snf1, β -subunit Gal83, and γ -subunit Snf4. Each subunit deletion strain (*snf1* Δ , *gal83* Δ , and *snf4* Δ) displayed reduced thermotolerance, distinct responses to antifungal drugs and oxidative stresses. The phenotypic recovery was confirmed through red fluorescence protein (RFP)-tagged complementary strains. Fluorescent imaging revealed the cytoplasmic localization of the Snf1 complex. The study is ongoing with the construction of all double and triple mutants to validate the complex's functional dominance. Our work provides a comprehensive insight into the Snf1 complex carbon sensing regulatory mechanism of *C. neoformans*.

Unraveling Novel Nuclear Localization Sequences of Nuclear Effectors, MoHTR2 and MoHTR3, in *Magnaporthe oryzae*

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The rice blast fungus, *Magnaporthe oryzae*, secretes various effector proteins to subvert the host immunity. Nuclear effectors such as MoHTR2 and MoHTR3 play pivotal roles in suppressing the expression of host immunity-related genes by directly binding to the effector binding element of the gene promoter in the host cell nucleus. Nuclear effectors carry a nuclear localization sequence (NLS), a short peptide sequence that guides the nuclear import of nuclear effectors. While some functional studies on NLS of TAL effectors and RxLR effectors were conducted, research on the NLS motif of fungal nuclear effectors remains limited. We found 12 and 17 amino acid sequences constituting the NLS of MoHTR2 and MoHTR3, respectively. Moreover, we revealed that MoHTR2 and MoHTR3 NLSs are non-classical NLSs that do not directly interact with rice importin alpha to transport into the nucleus. Further research will be performed to elucidate the core amino acids in the NLS required for successful nuclear localization and their potential role in the virulence of *M. oryzae* and modulation of host immunity-related genes. This comprehensive investigation seeks to contribute valuable insights into understanding the molecular mechanisms of fungal nuclear effectors.

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Rad6/Bre1 of Histone H2B Ubiquitination Machinery Regulates Morphogenesis in *Candida albicans*

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Candida albicans is an opportunistic human fungal pathogen. *C. albicans* normally exists as a normal flora, but when specific signals or stressful situations occur, it forms hyphae and infiltrates tissues to cause infection. The transition from yeast to hypha regulates the virulence of *C. albicans* and is caused by changes in the expression of many genes. Histone modifications can change in expression of genes, but it is unclear which histone modifications regulate the pathogenicity of *C. albicans*. In our study, we observed that the deletion of Rad6 or Bre1 of the histone H2B ubiquitination machinery, attenuated virulence in a mouse infection model. In contrast to what we had expected, we found that both mutant strains produced hyphae more quickly than the wild-type strain. Our results imply that processes other than filament formation are responsible for the attenuation of virulence in the absence of histone H2B ubiquitination.

The Histone Transcription Regulator Fgwd060 is Required for Fungal Development in the Plant Pathogenic Fungus *Fusarium graminearum*

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The fundamental structural unit of eukaryotic chromatin, the nucleosome, consists of genomic DNA wrapped around a core (“octamer”) of eight histone proteins (two each of histones H2A, H2B, H3, and H4). The expression of histone genes is strictly regulated to maintain a proper DNA-histone ratio and intact chromatin structure. Regulation of histone gene expression occurs at the transcriptional and post-transcriptional level; transcriptional regulatory mechanisms has not been well understood in filamentous fungi including fungal plant pathogens. Here, we show that Fgwd060 containing a WD40-domain is a crucial component of the hierarchical regulatory mechanism of histone gene transcription in *Fusarium graminearum*, a major causative agent of Fusarium head blight in small-grain cereals worldwide. Deletion of *FgWD060* resulted in relatively higher transcript levels of histone genes and pleiotropic defects in hyphal growth, conidiation, and sexual reproduction. Furthermore, this mutant produced markedly reduced amounts of mycotoxins such as trichothecenes and was highly sensitive to various stress conditions compared to the wild-type strain. Our results suggest that overproduction of the histone proteins led to impairment of chromatin assembly, causing various phenotypic defects in the absence of *FgWD060* in *F. graminearum*.

Uncovering *in planta*-specific Transcriptional Mechanism of a Fungal Effector Gene, *MoHTR1*, in the Rice Blast Fungus

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During plant-phytopathogen interactions, fungi secrete effector proteins into the host tissues to disrupt the immune system or adjust host metabolism for successful infection. Although many effector genes are expressed *in planta* stage, there is only limited information on the mechanisms of regulating *in planta* expression of effector genes. To understand the *in planta* specific expression of effector genes, we characterized the promoter of a nuclear effector gene of *Magnaporthe oryzae*, *MoHTR1*. To identify the IPA elements (*In planta active* elements) regulating transcription of *MoHTR1*, we performed a truncation analysis in the promoter region of *MoHTR1* with *sGFP* tagging. By comparing the fluorescence intensity to determine the promoter activity, we found the 8 bp of IPA element (TATTTTCGT). Mutation of the IPA element led to reduced virulence of the fungal pathogen as much as the deletion of *MoHTR1*. Of 156 genes containing the same IPA element sequences of *MoHTR1*, we unveiled that *in planta* specific expression of *Slp1* is also regulated by the same IPA element of *MoHTR1*. Furthermore, we found that *MoHTR1*'s promoter including the IPA element can induce *in planta* expression of *MobZIP14*, a transcriptional factor gene expressing during vegetative growth but not in the infection stage. We are now identifying transcriptional factor(s) binding to this IPA element of *MoHTR1* using pull-down assay, yeast one hybrid, and Luciferase assay. Our research will provide comprehensive insights into the regulatory mechanism of *in planta* specific expression of fungal effector genes.

Anti-prion System in *Saccharomyces cerevisiae*: Innate Immunity to Inside-the-cell Risk

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Prions are infectious proteins consisting with only of proteins. Prion conversion occurs when a prion-forming protein, normally functional, misfolded and later converted its structure to a prion form, not functional. Prions have been found in a wide variety of organisms. Yeast and their prions have used as a model system to study its pathological effect to the cell.

[PSI⁺] prion is filamentous polymers (amyloids) of Sup35p/eRF3, whose normal function is in translation termination. The conversion of Sup35 to Prion form results read-through of stop codon. By this reason, this [PSI⁺] and most of other prions are obviously detrimental to host cell. Unlike other infectious agents, these yeast prions can occur at any time in the cell in the presence of prion-forming genes. So, yeast has evolved the anti-prion system to limit inside-the-cell risk.

In *S. cerevisiae*, at least eight anti-prion systems deal with pathogenic amyloid yeast prions by (1) blocking their generation, (2) curing most variants as they arise, and (3) limiting the pathogenicity of prion variants that do arise and propagate.

In this study, we identified possible anti-prion components (JJJ1, RPL6B, TSR3) through screening using yeast knockout collection. As a result of prion induction by overproducing the Sup35 prion domain resulted in more than 10-fold increase in prion generation compared to WT. Further works will confirm that it is indeed a component of novel anti-prion system.

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A Genetic Based Deep-screening Target on Anti-[PSI+] Prion Component in *Saccharomyces cerevisiae*

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Prion is an infectious protein, mostly replicating pathogenic self-propagatable amyloid. 30 years of study about prions in yeast revealed that yeast has more than 10 prions such as [PSI+] (prion form of translation termination factor Sup35/eRF3), [URE3] (prion form of nitrogen catabolism regulator Ure2p) and [PIN+] (prion of functionally unknown Rnq1p), respectively. However, yeast also has evolved an array of anti-prion system targeting on these prions. Components of the systems are working constantly in a normal cell, blocking prion formation and propagation. To complement previous genetics-based screening, a deep-screening for finding possible anti-[PSI+] prion component(s) was performed using Yeast Knockout collection. RPS18A, RPL24B and RPL16A were screened and further investigated the effect of absence of these genes on prion formation frequency. Further works should include whether the restoration of each gene by mating, cytoduction and plasmid transformation can eliminate prion arising in absence of each. These will confirm that screened factors are indeed anti-[PSI+] components working in the normal WT cells like human innate immunity system.

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Discovery of Morphological Characteristics, Drug-resistance, and Pathogenicity Correlations of *Non-albicans Candida* Isolates

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In the One-Health concept, the most important medium connecting our surroundings with 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. Opportunistic infections may increase as drug-resistant microorganisms enter urban area. Therefore, we first isolated fungi from various sources such as patients, animal feces, and groundwater, and then identified the species using *ITS1* sequencing. In this study, we collected various strains of non-*albicans Candida* species from animal feces. As a result of comparing the antifungal susceptibility of the isolated strains, it was found that the MIC value of non-*albicans Candida* species was relatively higher than that of *Candida albicans* type strain. Interestingly, we observed that morphological changes in the strains with microscope were also inhibited after antifungal drug treatment. In future studies, we will analyze the mechanisms through which changes in hyphal morphology in yeast, specifically in relation to true- or pseudo-hyphal growth. Hyphal transition is a major virulence factor in *C. albicans*, but it has lower intrinsic drug resistance compared to non-*albicans* species that do not have true-hyphal growth. We will compare virulence and treatment options in a murine model of candidiasis. The goal is to identify genes associated with morphological traits that influence drug-resistance and pathogenicity.

Unveiling the Genome Diversity of *Magnaporthe oryzae* Using Pan-genome Analysis

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Magnaporthe oryzae is one of the most important plant pathogens that causes severe disease throughout the members of the Poaceae family. As different isolates of the fungal pathogen adapt to different environments such as its host plant, many dispensable genomes may emerge only in a particular subset of strains, which are not present in the reference genome. We used 435 *M. oryzae* genomes from isolates collected globally from diverse hosts to establish a pan-genome. We discovered that 5,706 of the 37,986 orthologs were core genes, present in all *M. oryzae* isolates. The remaining 32,280 orthologs were accessory genes, with its presence varying between each isolate. Furthermore, we observed that the isolates were clustered according to their host species at the intraspecific level. Characterizing the genome diversity of *M. oryzae* can give insight into how a fungal pathogen can diversify its genome makeup to adapt to different hosts.

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Identification of Essential Genes for the Establishment of Spray-induced Gene Silencing-based Disease Control in *Fusarium graminearum*

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The plant pathogenic fungus *Fusarium graminearum* causes Fusarium head blight (FHB) in major cereal crops such as wheat, barley, and rice, resulting in significant economic losses. As resistance to chemical fungicides continues to increase in *F. graminearum*, there is a growing need to develop novel disease control strategies. To discover essential genes that could serve as new disease control targets, we selected essential gene candidates that had failed to be deleted in previous studies. Thirteen genes were confirmed to be essential, either by constructing conditional promoter replacement (CPR) mutants or by employing a clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9)-mediated editing strategy. We synthesized double-stranded RNAs (dsRNAs) targeting these essential genes and analyzed their protective effects in plants using a spray-induced gene silencing (SIGS) method. When dsRNAs targeting *Fg10360*, *Fg13150*, and *Fg06123* were applied to detached barley leaves prior to fungal inoculation, disease lesions were greatly reduced. Our findings provide evidence of the potential of essential genes identified by a SIGS method to be effective targets for the control of fungal diseases.

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Investigation of Azole Resistance Mechanism through Genome-wide Association Analysis of *Aspergillus flavus* Strains

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Fungal disease control is critical to both the global economy and public health. *Aspergillus flavus* is dangerous to those who are infected because it produces aflatoxin, which harms both plants and animals. Chemical therapy that inhibits the formation of ergosterol (Azoles) is commonly used to treat *A. flavus*. However, the progressive development of fungicide resistance diminishes efficacy, resulting in overuse or harmful effects. In the current study, 123 strains of *A. flavus* obtained from various environments were tested for fungicide (metconazole) susceptibility, and mutations in the drug target genes (*cyp51s*) of all strains were examined. In addition, the population genomes of the selected 54 strains were analyzed to look for new resistance mechanisms. Based on the MIC value of 10 mg/L, the 54 strains were separated into two groups: less-sensitive (28 strains) and more-sensitive (26 strains). Following whole genome sequencing (NGS), Bcftool and snpEFF identified variants (SNPs/indels). The groups (K=2 to 6) examined by ADMIXTURE's maximum likelihood estimate process were shown. Using the Tessel5 program, the difference between the two groups for all variants was statistically assessed (P-value), and 12,469 exon-variants satisfying $P < 0.05$ were discovered out of 561,952 variants. Exon variations were functionally annotated, and genes with a wide range of domains, including the Major facilitator superfamily (MFS) domain and the Transcription factor (TF) domain, were discovered. Currently, a knock-out transformation strategy based on the CRISPR-Cas system is being investigated to determine whether gene function is associated with drug resistance. Our findings will increase the potential for future drug development and enable effective control of fungal pathogens beyond those currently used.

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Characterization of *SsHog1* and *Shk1* Involved in Fungicide Resistance, Osmotic Stress, and Virulence by Repeated Protoplasting and RNP Delivery Method in *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum is the causal agent of sclerotinia stem rot on over 400 plant species. To manage the disease, fungicides including dicarboximides and phenylpyrroles have been widely applied. It is known that the class III histidine kinase gene (*Shk1*) is involved in iprodione and fludioxonil sensitivity, and osmotic stress in *S. sclerotiorum*. To further investigate the fungicide sensitivity associated with the high-osmolarity glycerol (HOG) pathway, the putative last kinase *SsHog1* gene was characterized. The genetically pure homokaryotic *SsHog1* and *Shk1* knockout mutants were generated using split marker transformation combined with a newly developed repeated protoplasting method and CRISPR/Cas9 ribonucleoprotein (RNP) delivery approach. Our results demonstrate that *SsHog1* is involved in hyperosmotic adaptation, fungicide resistance, and pathogenicity. Moreover, we suggest that the repeated protoplasting method is an applicable option for more accurate gene functional characterization of multinucleate fungi.

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Structural and Functional Characterization of *N*-/*O*-Glycans Assembled on Mannoproteins MP88 and Chitin Deacetylase 1 in the Human Fungal Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is an opportunistic fungal pathogen causing life-threatening disease in immunocompromised individuals. Several Cryptococcal mannoproteins (MPs), such as MP98 and MP84, are reported as key antigens stimulating host CD4 (+) T-cell response. In this study, we delineated the structures of *N*-/*O*-glycans of MPs, focusing on MP88 and chitin deacetylase 1 (CDA1), and investigated the effect of altered glycan structures on the interaction with host cells. CDA1 converts chitin into chitosan, which is essential for the cell wall integrity, and MP88 is a major protein associated with extracellular vesicles of *C. neoformans*. The His-tagged MP88 protein was secreted by deleting GPI anchor motif from the acapsular *C. neoformans alg3D* strain with a defect in *N*-glycosylation. The purified MP88(H) with truncated core-*N* glycans stimulated more efficiently several cytokine secretion of the dendritic cells compared to the WT-secreted MP(H)s. The MP88(H) and CDA1(H) proteins with *O*-glycans with and without xylose, respectively, were generated by secretion from the acapsular the *C. neoformans* strains *ktr3D* and *cap6D* with defects in *O*-mannosylation. The purified CDA1 and MP88 proteins with the altered *N*-/*O*-glycan structures will be valuable tools to examine the relationship between glycan structure and function in adhering to host cells and inducing the cytokine secretion of host cells. Particularly, MPs carrying exclusively *O*-glycans with or without xylose residues could provide the insights into the role of xylose conjugated *O*-glycan glycan in the interaction with host cells. The information generated by this study would be useful for the development of next generation antifungal drugs based on fungal-specific glycans.

Genomic and Physiological Characterization of Yeast Species Isolated from Korean Rice Wine and Vinegar

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In this study, we identified and characterized yeast strains isolated from various kinds of Makgeolli and vinegar on the market. ITS and whole-genome sequence analyses identified all six yeast isolates from Makgeolli as *Saccharomyces cerevisiae*, although four of them were closely related to *Saccharomyces paradoxus* in the phylogeny analysis, whereas one yeast isolate from vinegar as *Zygosaccharomyces bailii*. Notably, the Makgeolli CS and KSD strains showed higher ethanol tolerance than the reference *S. cerevisiae* strain, and the vinegar *Z. bailii* strain showed the highest acetic acid tolerance among tested several yeast species. Ploidy analysis revealed that all the Makgeolli strains were diploid, while the vinegar strain was haploid. Headspace-SPME GC-MS analysis showed that three of the six Makgeolli strains were not able to produce 4-vinylguaiacol (4-VG), an essential clove-like flavor for some alcoholic beverages, due to the presence of nonsense mutation in their *FDC1* gene, encoding ferulic acid decarboxylase involved in conversion of ferulic acid to 4-VG. Interestingly, *Z. bailii* exhibited a unique volatile aroma profile with significantly much higher levels of a few flavor compounds, benzaldehyde and phenethyl butyrate, compared to *S. cerevisiae* and *Zygosaccharomyces rouxii*. This integrated study on the genomic and physiological properties of yeasts will contribute to improving the flavor and functionality of Korean traditional fermented beverages and condiments.

The Function of a Myb-like Protein MylA in *Aspergillus flavus*

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The representative filamentous fungi, *Aspergillus*, proliferate by producing conidia (asexual spores), and the procedures of conidia production (conidiation) are delicately regulated by a variety of transcription factors. Among them, Myb-like transcription factors (TFs) are one of the large TF family and modulate the levels of transcription or translation of their targets by sequence-specific DNA binding activities and protein-protein interaction activities. In our previous study, we identified 5 Myb-like (Myl) proteins that are highly expressed in the spore of *Aspergillus nidulans*. The deletion mutants (*mylA*^{~E}) were generated and among them, the deletion of *mylA* showed impaired growth and conidiation in *A. nidulans* and *A. fumigatus*. To further characterize the conserved role of MylA among *Aspergillus* species, the *mylA* deletion mutant was generated in plant pathogenic fungus *Aspergillus flavus*, and phenotypic analysis was carried out. The *mylA*-deficient mutant showed reduced conidia production and colony growth compared to the control. Also, the *mylA* strain produced a reduced amount of sclerotia. Next, we analyzed the role of MylA in *A. flavus* conidia and found out deletion of *mylA* resulted in reduced trehalose content, spore viability, and stress tolerance in *A. flavus*. Lastly, we assessed the function of MylA in *A. flavus* pathogenicity by performing a kernel bioassay. When incubated with kernels, the *mylA* null mutant showed reduced conidia colonization and aflatoxin B1 production. Taken together, these results suggest that MylA plays pivotal roles in proper fungal development, conidial viability, and pathogenicity of *A. flavus*, and the functions of MylA in conidia are conserved in three *Aspergillus* species.

Seven Unrecorded Fungi Isolated from Fire Blight-controlled Apple Orchard Soil in Korea

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Fungal diversity in orchard soil where fire-blighted apple trees are buried is important to understand the decay process of fire-blight-controlled apple trees. To explore fungal diversity in orchard, we collected soil samples from apple orchards in Chungju, Korea. Fungal isolates were cultured on PDA, MEA, CYA, and OA for morphological analysis. The PCR-amplified products of the ITS1-5.8S-ITS2 region (ITS1, 4) as well as 28S large subunit of the nuclear ribosomal RNA gene (LSU), partial sequences of the β -tubulin, calmodulin, and translation elongation factor 1- α genes (TEF1- α) were sequenced and analyzed phylogenetically for molecular identification. Seven previously unknown fungal species in Korea were identified as *Aspergillus aureolatus*, *Botryotrichum atrogri-seum*, *Dactylonectria novozelandica*, *Fusarium denticulatum*, *Paecilomyces tabacinus*, *Sarcopodium tibetense* and *Talaromyces stollii*. This study is expected to contribute to basic data for environmental research on orchard soil and saprophytic soil fungal diversity.

Five New Species of Nectriaceae Isolated from Various Soil Samples

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The Nectriaceae family, circumscribed by brothers Charles and Louis René Tulasne in 1865, refers to fungi that form uniloculate perithecia that are generally orange-red to purple under KOH or yellow under 100% lactic acid conditions. The characteristic feature of the asexual morph of Nectriaceae is that it forms amerosporous to phragmosporous conidia. Nectriaceae has high species diversity, with generally higher species diversity in warm temperate and tropical regions. Wijayawardene reported that by 2022, 70 genera, including *Gliocladiopsis*, *Ilyonectria*, *Mariannaea*, and *Volutella* belong to Nectriaceae. We collected soil samples from Iseung-ak Oreum of Jeju Island, Upo wetland of Changnyeong-gun and Seopjikoji of Jeju Island. Soil samples were diluted, spread on dichloran glycerol 18% agar, and cultured in an incubator at 25°C for 2 weeks. The growing fungal colonies were isolated on potato dextrose agar and cultured in an incubator at 25°C for 2 weeks. The isolated fungal strains were identified through molecular genetic analysis by analyzing the maximum likelihood phylogenetic tree based on ITS, LSU, *BenA*, *TEF-1 α* , and *HIS3* sequences. One species of *Gliocladiopsis*, one species of *Ilyonectria*, one species of *Mariannaea*, and two species of *Volutella* were identified as new species belonging to Nectriaceae. The microstructures of the identified novel fungal strains were observed using an optical microscope. In this presentation, we present the morphological and molecular phylogenetic tree results for the five novel species.

The Protein Phosphatase 2C Domain Contributes to the Pathobiological Function of Adenylyl Cyclase, Cac1 in *Cryptococcus neoformans*

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The cAMP signaling pathway governs growth, differentiation, stress response and adaptation, and the pathogenicity of plant and animal fungal pathogens. The central component of the cAMP pathway is adenylyl cyclase (AC), which produces cAMP in response to external signal and subsequently activates protein kinase A (PKA) to regulate its downstream effector proteins. The AC consists of multiple protein domains, including Ga-binding domain, Ras-associated domain, leucine-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*^{PPΔ} and *CAC1*^{ACΔ} 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. Surprisingly, however, we found that the PP2C domain plays a partial role in melanin and capsule production. In conclusion, here we demonstrate that the PP2C domain contributes to the function of Cac1 in virulence factor regulation of *C. neoformans*.




Participants

The 25th Fungal Genetics and Biology Conference of the Microbiological Society of Korea, 2024

2024년 제 25차 학술대회 참가자 명단

Participants in the 25th Korean Fungal Genetics & Biology Conference, 2024

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(2023, The 24th Korean Fungal Genetics and Biology Conference, Deoksan)

제25회
**진균유전생물
컨퍼런스**

