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光滑念珠菌 SAGA 複合體於藥物耐受性及毒力

之角色探討

The roles of SAGA complex in drug tolerance and
virulence in *Candida glabrata*

楊聖永

Sheng-Yung Yang

指導教授：陳穎練 博士

Advisor: Ying-Lien Chen, Ph.D.

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本論文係楊聖永君（R06633009）在國立臺灣大學植物病理與微生物學所完成之碩（博）士學位論文，於民國 108 年 11 月 25 日承下列考試委員審查通過及口試及格，特此證明

口試委員：

陳穎練 博士 陳穎練 (指導教授)

藍忠昱 博士 藍忠昱
國立清華大學分子與細胞生物研究所 教授

陳宜君 醫師 陳宜君
國立台灣大學醫學院內科 教授

羅秀容 博士 羅秀容
國家衛生研究院感染症與疫苗研究所 研究員

系主任、所長 洪挺軒 (簽名)



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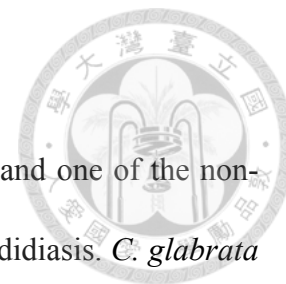
中文摘要



光滑念珠菌為一種人體伺機性病原真菌，可附著在黏膜組織上並侵入血管造成系統性感染，其先天性耐藥性造成臨床治療上的困難。目前光滑念珠菌對於抗真菌藥物的耐受性、氧化壓力及毒力調控機制尚未有完整研究。SAGA 複合體藉由乙醯化組蛋白調控基因的表現，其組蛋白乙醯化模組主要由 Ada2、Ada3 及 Gcn5 組成。本實驗室之前人研究發現光滑念珠菌 *ada2* 突變株對於抗真菌藥物及干擾細胞壁之化合物的耐受性會下降，但此突變株之毒力卻增強。為了瞭解 Ada3 和 Gcn5 是否與 Ada2 有相似或是相異的功能，我們利用剔除個別基因、雙基因及三基因之突變株進行實驗。結果顯示單一基因 (*ADA3* 或 *GCN5*)、雙基因以及三基因剔除之突變株中，組蛋白 (H3K9) 的乙醯化程度有顯著下降、生長速率較野生株緩慢、細胞壁的完整性下降、對於抗真菌藥物及氧化壓力的反應更敏感。有趣的是 *gcn5* 突變株對於影響細胞壁完整性之化合物、氧化壓力以及抗真菌藥物的反應較 *ada2* 或 *ada3* 突變株稍佳。此外，所有突變株入侵洋菜膠的能力增強且可促進毒力相關基因之表現。在小鼠系統性感染實驗中，*ada3* 和 *gcn5* 突變株接近高毒力，然而 *ada3 gcn5* 雙基因突變株呈現高毒力。同時，雙基因突變株 (*ada2 ada3* 和 *ada2 gcn5*) 以及三基因突變株 (*ada2 ada3 gcn5*) 亦表現高毒力。總結，Ada3 及 Gcn5 扮演與Ada2 相似的角色，參與調控 H3K9 乙醯化、氧化壓力反應與藥物耐受性，然而對於洋菜膠的侵入能力或是毒力卻是扮演抑制的功能。

關鍵字：光滑念珠菌、SAGA 複合體、藥物耐受性、氧化壓力、毒力

Abstract



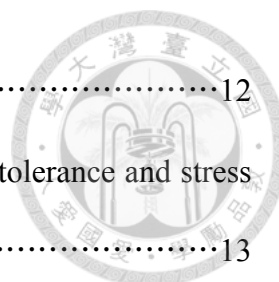
Candida glabrata is an opportunistic human fungal pathogen and one of the non-*albicans* *Candida* species frequently isolated from patients with candidiasis. *C. glabrata* has intrinsic tolerance to antifungal drugs and ability to adhere on mucocutaneous surfaces, invade into bloodstream and cause systemic infection. However, the regulation of drug tolerance and virulence of *C. glabrata* remains elusive. SAGA (Spt-Ada-Gcn5 acetyltransferase) complex controls gene expression by regulating histone acetylation through the histone acetylation module (HAT) Ada2-Ada3-Gcn5. Our previous study showed that *ada2* mutant is hypervirulence but decreases tolerance to antifungal drugs (i.e., azoles, echinocandins and polyene) and cell wall perturbing agents (i.e., calcofluor white, Congo red and SDS). This study further characterizes the functions of Ada3 and Gcn5 in *C. glabrata*. We found that *ada3*, *gcn5*, double or triple mutants in HAT module resulted in decreased level of acetylation on H3K9, slower growth, decreased antifungal drugs tolerance and oxidative stress response, while *gcn5* mutant exhibited intermediate growth between the wild type and *ada2* or *ada3* mutant. In addition, HAT mutants increased agar invasion and expression of virulence associated genes. The *ada3* and *gcn5* mutants exhibited marginal hypervirulence, while double mutant (*ada3 gcn5*) showed hypervirulence in a murine model of systemic infection. Meanwhile, HAT double mutants (*ada2 ada3* and *ada2 gcn5*) and triple mutant (*ada2 ada3 gcn5*) were hypervirulence. In conclusions, *C. glabrata* Ada3 and Gcn5 play similar roles as Ada2, regulating acetylation of H3K9, drug and oxidative stress tolerance and virulence.

Keywords: *Candida glabrata*, SAGA complex, drug tolerance, oxidative stress, virulence

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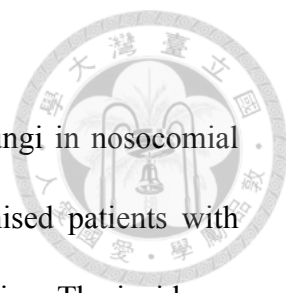
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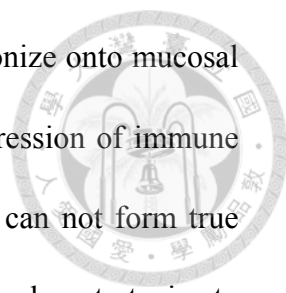
1. Introduction



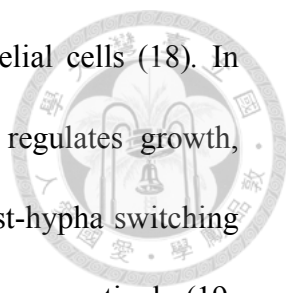
Candida species are one of the major cause of pathogenic fungi in nosocomial infection with high morbidity and mortality in immunocompromised patients with organ transplantation, iatrogenic immunosuppression or HIV infection. The incidence of candidiasis is 3-5 per 100,000 persons and the mortality can reach to 10-20% (1). In recent years with improved identification methods and intervention of drug, more patients were infected by non-*albicans Candida* species (NACs) rather than *Candida albicans*. In Taiwan, *Candida tropicalis* is the most frequently isolated NAC in clinical practice. Instead, *C. glabrata* is the most prevalent species among NACs from patients with candidiasis in most of countries (1, 2). The difference between countries is usually depending on the geographic regions, patients group and drug usage. However, *C. albicans* is still the most prevalent species in clinical isolates. Consequently, extensive studies have already focused on virulence factors of *C. albicans* but how NACs threat to our health remain elusive. In addition, NACs were considered possessing the intrinsic antifungal drug resistance, indicating that our treatments are losing the ability to combat candidiasis efficiently.

Azoles, echinocandins and polyenes are three different actions of antifungal drugs, usually used in the first line of candidiasis treatment. However, the numbers of *Candida* species that have intrinsic antifungal drug resistance are increasing in decades which can be attributed to inherent problem that it is hard to find an effective target without being toxic to human (3, 4). Without a new strategy or target, it may facilitate the rising incidence of drug resistant isolates.

C. glabrata is an opportunistic fungal pathogen with intrinsic drug tolerance and



acts as a commensal organism under normal conditions. It can colonize onto mucosal membrane and skin and further invade to bloodstream during depression of immune system, causing inflammation of multi organs. Since *C. glabrata* can not form true hyphae as *C. albicans* does to get rid of immune cells, it must develop strategies to withstand harsh environment given by macrophages or neutrophils. To rapid response to the changing environment, regulations of gene expressions in a manner of histone modification plays a crucial role in *C. glabrata* (5, 6). Histone modification includes acetylation, ubiquitination, phosphorylation, methylation and sumoylation. Within histone acetylation, Gcn5 is a catalytic subunit of SAGA (Spt-Ada-Gcn5 acetyltransferase) complex that has been well studied in *Saccharomyces cerevisiae*, responsible for acetylation on lysine residue of histone. SAGA complex is a multifunctional gene regulator that comprises about 20 subunits, including histone acetylation module, TATA-box binding protein (TBP), cofactor for RNA polymerase II and deubiquitination module. Histone acetyltransferase (HAT) module can transfer an acetyl group from acetyl-CoA to a lysine residue, which can neutralize the positive charge of lysine and facilitate gene transcription (7-10). In previous studies, *S. cerevisiae* Ada2 and Ada3 play a role as adapter, assisting the catalytic subunit, Gcn5, to transfer an acetyl group specifically as well as to enhance the acetyltransferase efficiency (11, 12), while Gcn5 bromodomain is contributed to site specificity of H3 lysine residue acetylation (13). In addition to the function in specific lysine acetylation, Ada2, Ada3 are involved in rapid response to glucose for G₁ cyclin induction (14) and Gcn5 is essential for respiration and DNA replication (15, 16). Besides, *S. cerevisiae* ADA2 and GCN5 are required for the transcription of FLO1 (17) which ortholog in *C.*




glabrata is *EPAl*, controlling the adhesion of yeast to host epithelial cells (18). In pathogenic yeasts, the acetyltransferase within SAGA complex regulates growth, adaption to various environment and virulence factor, such as yeast-hypha switching and capsule formation of *C. albicans* and *Cryptococcus neoformans*, respectively (19-22). Meanwhile, in filamentous fungus, *Aspergillus fumigatus* GcnE regulates conidiation and biofilm formation but not contributes to virulence in murine model (23). Our previous study shows that deletion of *ADA2* increases the drug tolerance and virulence in *C. glabrata* but the roles of *Ada3* and *Gcn5* in drug tolerance and virulence remain elusive (24, 25).

In present study, we demonstrate that deletion of *ADA3* or *GCN5* decreased the acetylation level of H3K9, growth rate, drug tolerance and cell wall integrity, indicating conserved roles as *ADA2* in *C. glabrata*. Our results revealed new findings that *Ada3* and *Gcn5* involve in oxidative stress response. Interestingly, deletion of *GCN5* has an intermediate effect to cell wall perturbing agents and reactive oxygen species compared to wild-type (CBS138) and *ada2* or *ada3* mutant. Deletion of *ADA3* or *GCN5* increased agar invasion, adhesion related gene expression and led to marginal hypervirulence while deletion both *ADA3* and *GCN5* resulted in hypervirulence in murine systemic infection model.

2. Materials and Methods

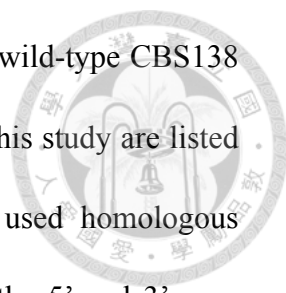
2.1 Strains, media and chemicals



C. glabrata strains used in this study are listed in Table 1. Yeast-peptone-dextrose (YPD, 1% yeast extract, 2% peptone, 2% glucose) liquid and agar (2%), synthetic complete (SC) medium (0.17% yeast nitrogen base without amino acid, 0.5% (NH₄)₂SO₄, 2% glucose, amino acids, and 2% agar), Luria-Bertani (LB, 1% tryptone, 0.5% yeast extract, 1% NaCl) liquid and agar (2%) were used in this study. YPD containing 100 µg/mL nourseothricin (Werner BioAgents, Jena, Germany) was used to select *C. glabrata* transformants. LB containing 34 µg/mL chloramphenicol (BioShop, Burlington, ON, Canada) or Ampicillin (BioShop, Burlington, ON, Canada) was used to select *E. coli* transformants. Sodium dodecyl sulfate (SDS; Bioman, New Taipei city, Taiwan), calcofluor white (CFW; fluorescent brighter 28, Sigma, St. Louis, MO, USA), Congo red (CR; Genzyme, Cambridge, MA, USA), fluconazole (FLC; selleckchem, Houston, TX, USA), posaconazole (PSC; Merck, Rahway, NJ, USA), voriconazole (VRC; sigma), micafungin (MCF; Astellas Pharma Inc., Deerfield, IL, USA), caspofungin (CSF; Merck), anidulafungin (ANF; Pfizer Inc., Groton, CT, USA) and amphotericin B (Sigma, St. Louis, MO, USA) were added to the media at the concentrations indicated below. Minimum inhibitory concentration was performed using Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, St. Louis, MO, USA) buffered with MOPS (Sigma, St. Louis, MO, USA).

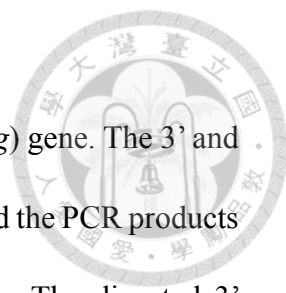
2.2 Gene disruption and complementation in *C. glabrata*

All the disruption cassettes and complementation cassettes were made by Shang-



Jie Yu. All deletion mutants were generated from the prototrophic wild-type CBS138 (26) using the *SAT1*-flipper system (27). All the plasmids used in this study are listed in Table 2. To disrupt the *GCN5* (*CAGL0F08283g*) gene, we used homologous recombination method. We first amplified approximately 1kb of the 5' and 3' non-coding region (NCR) of the *GCN5* open reading frame (ORF) with primer JC717/JC718 (for 3' NCR) (Table 3) and JC715/JC716 (for 5' NCR) from the genomic DNA of the wild-type CBS138. The PCR product of 3' and 5' *GCN5*^{NCR} were double digested with two restriction enzymes *Sac*II/*Sac*I and *Kpn*I/*Apa*I, respectively. The digested 3' *GCN5*^{NCR} PCR product was purified and cloned into plasmid pSFS2A, resulting in plasmid pYSJ59. The digested 5' *GCN5*^{NCR} PCR product was then purified and cloned into pYSJ59 to make the *GCN5* disruption plasmid pYSJ61. The *GCN5* disruption cassette 5' *GCN5*^{NCR}-*SAT1-FLP*-3'-*GCN5*^{NCR} was excised from pYSJ61 using restriction enzymes *Kpn*I and *Sac*I, then transformed by modified lithium acetate transformation method (28) into wild-type CBS138 to obtain the nourseothricin-resistant *gcn5* mutant YSJ65. To generate *GCN5* complementation strain, we first removed the nourseothricin-resistant marker *SAT1-FLP* from *gcn5* mutant YSJ65 by culturing YSJ65 in YPD medium for 4 days then spread on YPD plates for two days and replicated colonies onto nourseothricin-containing plate to confirm the loss of *SAT1-FLP*, resulting in nourseothricin-sensitive *gcn5* mutant YSJ104. Second, we amplified 5' *GCN5*^{NCR} – *GCN5* from CBS138 genomic DNA using primer JC1307/JC1308, double digested with *Xho*I and *Hind*III and cloned into pYSJ59 make plasmid pYSJ101. The complementation cassette 5' *GCN5*^{NCR}-*GCN5-SAT1-FLP*-3' *GCN5*^{NCR} was excised from pYSJ101 using *Xho*I and *Sac*I and transformed into

YSJ104 to obtain the *GCN5* complementary strain YSJ108.



A similar approach was used to disrupt *ADA3* (*CAGL0E00693g*) gene. The 3' and 5' NCR of *ADA3* were amplified with JC906/907 and JC904/905, and the PCR products were double digested with NotI/SacI and ApaI/XhoI, respectively. The digested 3' *ADA3*^{NCR} PCR product was purified and cloned into plasmid pSFS2A, resulting in plasmid pYSJ58. The digested 5' *ADA3*^{NCR} PCR product was then purified and cloned into pYSJ58 to make the *ADA3* disruption plasmid pYSJ63. To generate *ada3* mutant, we digested pYSJ63 with ApaI and SacI to release the disruption cassette and transformed into the wild-type CBS138, resulting in *ada3* mutant (YSJ68). The *SATI-FLP* was removed from YSJ68 using the same approach mentioned above to obtain nourseothricin-sensitive *ada3* mutant, YSJ115. To make complementation cassette, we amplified 5' *ADA3*^{NCR}-*ADA3* from CBS138 genomic DNA using primer JC904/JC1309, double digested with ApaI and XhoI and cloned into pYSJ58 to make plasmid pYSJ103. The complementation cassette 5' *ADA3*^{NCR}-*ADA3*-*SATI-FLP*-3' *ADA3*^{NCR} was excised from pYSJ103 using ApaI/SacI and transformed into YSJ115 to obtain the *ADA3* complementary strain YSJ126.

For generating *ada2 ada3*, *ada2 gcn5* and *ada3 gcn5* double mutants, we used the nourseothricin-sensitive *ada2* mutant (YSJ43) from our previous study (29) and *ada3* mutant (YSJ115) in this study as background strains. The enzyme digested *ADA3* or *GCN5* disruption cassette from pYSJ63 or pYSJ61 was transformed into YSJ43 and YSJ115 respectively to obtain double mutants *ada2 ada3* (YSJ74), *ada2 gcn5* (SY26) and *ada3 gcn5* (SY20).

For generating *ada2 ada3 gcn5* triple mutant, we used the nourseothricin-sensitive

ada2 ada3 double mutant (YSJ131) which nourseothricin-resistant marker *SAT1-FLP* was removed from YSJ74 as background strain. The enzyme digested *GCN5* disruption cassette from pYSJ61 was transformed into YSJ131 to obtain triple mutant *ada2 ada3 gcn5* (SY16). All strains were confirmed by PCR amplification of ORF, 5' and 3' NCR integration of the disruption cassette.

2.3 Determination of H3K9 acetylation

The procedure of total protein extraction was modified from previous study (29). Briefly, cells were grown overnight at 30°C with shaking at 200 rpm, then adjust to 0.2 OD₆₀₀ with 50 mL fresh YPD broth and incubated for 4 h at 37°C with shaking at 200 rpm. Then cells were centrifuged, washed once with distilled H₂O and resuspended in 1 mL extraction buffer (25 mM Tris-HCl, pH7.4, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM β-mercaptoethanol, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride, 0.94 mM sodium orthovanadate). Total proteins were isolated using the glass beads method with 0.5 mm diameter glass beads, samples were homogenized five times for 30 seconds by vortex and placed on ice between each homogenization. Crude extractions were quantified using Bradford method. The 10 μg of proteins were separated on SDS-PAGE gels and transferred to nitrocellulose membrane. The membranes were blocked in 5% silk milk (Fonterra, Auckland City, New Zealand) in Tris-buffered saline with Tween 20 (TBST; 20 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) and incubated with primary anti-actin antibody (#MAB1501; Merck Millipore) or anti-H3K9ac antibody (#07-352; Millipore, Temecula, CA, USA) overnight at 4°C with shaking. The membranes were then washed

three times with TBST buffer and incubated with secondary horseradish peroxidase-conjugated goat anti-mouse IgG antibody (#AP124P; Merck Millipore, Billerica, MA, USA) or goat anti-rabbit IgG antibody (#ab205718; Abcam, Cambridge, MA, USA). Chemiluminescence signals were detected using an enhanced chemiluminescence system (T-Pro Biotechnology, New Taipei City, Taiwan).

2.4 Growth kinetics assay

Cells were grown overnight at 30°C with shaking at 200 rpm, then washed twice with ddH₂O and diluted to 0.01 OD₆₀₀ with 50 mL YPD broth. Optical measurements were conducted at 0, 3, 6, 9, 12, 24 and 48 hours at 37°C using SpectraMax 190 microplate reader. To calculate doubling times, cells were diluted to 0.01 OD₆₀₀ with 200 µL YPD broth and the optical measurements were monitored every 15 min during 48 hours at 37°C. The doubling times were calculated at exponential phase (3-6 h) and using the formula as $dT = \ln 2 (T2 - T1) / \ln OD2 - \ln OD1$. dT, OD2 and OD1 represents doubling time, optical density at final time (T2) and optical density at initial time (T1), respectively.

2.5 Serial dilution spotting assay

Cells were grown overnight at 30°C with shaking at 200 rpm, then washed twice with ddH₂O and diluted to 1 OD₆₀₀ with ddH₂O, then 3 µl of 5-fold serial dilution of cell suspensions were spotted onto YPD plate in the absence or presence of anti-fungal drug, reactive oxygen species (ROS) or cell wall perturbing agents, then incubated at 37°C for 24 or 48 h.



2.6 Agar invasion assay

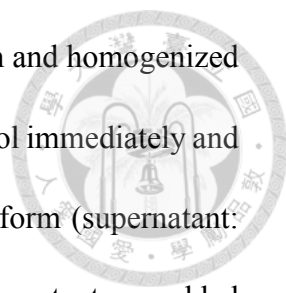
Cells were grown overnight at 30°C with shaking at 200 rpm, then washed twice with ddH₂O. The cell suspensions were diluted to 1 OD₆₀₀, 3 μL were spotted on YPD plate and incubated at 37°C for 3 days, then colonies were removed using swab and washed with dH₂O.

2.7 Determination of minimum inhibitory concentrations

To determine the minimum inhibitory concentrations, we followed CLSI guideline M27-A3 (30). Fluconazole (FLC), posaconazole (PSC), voriconazole (VRC), micafungin (MCF), caspofungin (CSF), anidulafungin (ANF) and amphotericin B (AmB) representing three different actions of antifungal drug were used in this study. Briefly, 100 μL of 2-fold serial diluted drugs and 100 μL cell suspensions were added into 96-well polystyrene plate. The final concentration of cell suspensions was 3×10^3 cells/mL. The plates were incubated at 35°C for 24 h without shaking. The quality control strain *Candida krusei* CBS573 was used to ensure that the drug, medium and procedures were reliable.

2.8 Real-time qRT-PCR

The procedure of RNA extraction was modified from previous study (29). Cells were grown overnight in YPD broth at 30°C with shaking at 200 rpm, then adjusted to 0.2 OD₆₀₀ with 50 mL fresh YPD broth containing 2 mM H₂O₂, incubated at 37°C for 3 h with shaking at 200 rpm. Cells were centrifuged at 3500 rpm for 10 min and poured



off the supernatant, cells were immediately placed in liquid nitrogen and homogenized with beads. After homogenization, cells were added with 1 mL TRIzol immediately and centrifuged at 4°C, 12,000×g. Supernatant was treated with chloroform (supernatant: chloroform = 5:1) then centrifuged for 10 min at 4°C, 12,000×g. Supernatant was added with isopropanol (v: v = 1: 1) and placed on ice for 10 min. Then centrifuged for 10 min at 4°C, 12,000×g. Pellet was washed with 200 μL 75% EtOH twice, then dried the pellet and resuspended with 200 μL DEPC-treated water. Using Turbo DNA-free kit (Invitrogen, Carlsbad, CA, USA) to eliminate DNA, then 1 μg DNA-free RNA were reverse transcribed to cDNA using high capacity reverse transcription kit (Thermo Fisher Scientific Baltics, UAB). 10 μL qPCR reaction mixtures included 2 μL cDNA (1 ng/μL), 5 μL 2× quantitative PCR master mix, 0.5 μL forward and reverse primers (5 μM) and 2 μL distilled water. Quantitative PCR running program were: 95°C 7 min for denaturation, 95°C 10 sec; 60°C 30 sec (40 cycles) and 95°C 15 sec; 60°C 60 sec and 95°C 15 sec (milting curve). StepOnePlus system and StepOne (v2.2) system were used to determine cycle threshold (C_T) value and the transcription level were quantified using 2^{-ΔΔC_T} method. *C. glabrata ACT1* was used to normalize the relative quantity. *P* values were determined using one way ANOVA.

2.9 Murine systemic infection model

Five to six-week-old male ICR mice (BioLasco Taiwan Co., Ltd.) were used in this study. 10 mice as one group and all groups were administrated with 150 mg/kg cyclophosphamide (Sigma, St. Louis, MO, USA) at day -3, 0 and 1. Cells were grown in liquid YPD overnight at 30°C with shaking at 200 rpm, washed twice with


phosphate-buffered saline (PBS), then adjusted to 3.5×10^8 cells/mL. 200 μ L (7×10^7 cells) cell suspension was used for systemic infection in a lateral tail vein injection manner. The course of infection was monitored for 15 days. Statistical analysis was conducted using Mantel-Cox log-rank test and the *P* value was used to determine the significant difference of virulence between wild type and HAT mutants.

2.10 Ethics statement

All experimental procedures were carried out according to NIH guidelines and were approved by the Institutional Animal Care and Use Committee at National Taiwan University (approval number NTU108-EL-106005).

3. Results

3.1 *Ada3* and *Gcn5* regulate H3K9 acetylation in *C. glabrata*



Chromatin-mediated expression is one of the strategies for *Candida* species to adapt the various environment. Changing the structure of nucleosome and distinct gene expressions are determined by specific lysine acetylation (31). In our previous study, deletion of *ADA2* in *C. glabrata* results in decreased acetylation level of H3K9 but not H3K14 (29). However, the roles of *C. glabrata ADA3* and *GCN5* in H3K9 acetylation are unknown. To test whether *ADA3* and *GCN5* are required for H3K9 acetylation, we performed western blots. Results showed that deletion of *ADA3* and *GCN5* decreased the H3K9 acetylation level (Fig. 1). We did not observe the difference of acetylated H3K9 among *ada2* mutant, *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*), indicating that the HAT module (*ADA3*, *GCN5* and *ADA2*) acts in concert to regulate H3K9 acetylation. Interestingly, H3K9 was still acetylated in triple mutant (*ada2 ada3 gcn5*), indicating that other components might also involve in H3K9 acetylation in *C. glabrata*.

3.2 *Ada3* and *Gcn5* are required for growth in *C. glabrata*

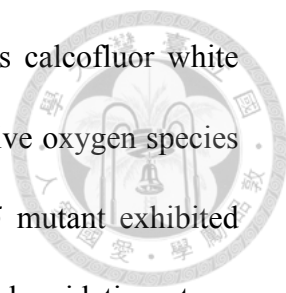
Approximately 65% of *Candida* species can not grow well at human body temperature (4). Loss of *ADA2* or *ADA3* in *S. cerevisiae* is sensitive to grow at 37°C (32), and the loss of *ADA2* in *Cryptococcus neoformans* and *C. glabrata* reduced the growth rate at 37°C (19, 29). It is unclear whether *C. glabrata ADA3* and *GCN5* are required for growth at 37°C. *C. glabrata* strains were incubated in YPD broth at 37°C and evaluated growth kinetics using SpectraMax 190 microplate reader. Loss of *ADA3*

or *GCN5* exhibited reduced growth rate compared to the wild type after 9 h ($P < 0.05$, two way ANOVA) (Fig. 2), similar reduced growth was observed in double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*). There is no significant difference among *ada3* mutant, *gcn5* mutant, double mutants and triple mutant. The *ada3::ADA3* and *gcn5::GCN5* complementary strains had a similar growth to the wild type.

3.3 *C. glabrata* Ada3 and Gcn5 play crucial roles in drug tolerance and stress responses

To investigate the roles of *ADA3* and *GCN5* in drug tolerance, we determined minimum inhibitory concentration (MIC) and performed serial dilution spotting assay. We found that *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*) decreased tolerance to azoles (FLC, PSC and VRC), echinocandin (MCF) and polyene (AmB) (Table 4). Interestingly, *gcn5* mutant exhibited intermediate viability under the stress from CSF compared to the wild type and other HAT mutants (Fig. 3). In addition to drug tolerance, the relationship between *ADA3/GCN5* and stress responses is still unclear in *C. glabrata*.

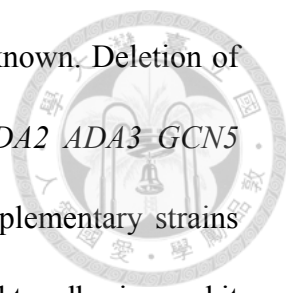
Three actions of antifungal drugs function at cell wall or components within the cell wall leading to osmotic instability and cell death. To determine whether deletion of *ADA3* or *GCN5* decreases the cell wall integrity and the response to oxidative stress, strains were serially diluted and spotted onto YPD in the absence or presence of cell wall perturbing agents or reactive oxygen species. We found that *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and triple mutant (*ada2 ada3*



gcn5) were more sensitive to cell wall perturbing agents, such as calcofluor white (CFW), Congo red (CR), sodium dodecyl sulfate (SDS), and reactive oxygen species (ROS), such as H₂O₂ and menadione (Fig 4). Interestingly, *gcn5* mutant exhibited intermediate responses under the cell wall perturbing agents and oxidative stress compared to the wild type and other HAT mutants. Gene encoding superoxide dismutase (SOD), catalase (CTA) or glutathione peroxidase (GPX) are three major actions to detoxify the ROS. To clarify which gene was contributed to oxidative response regulated by *ADA3* and *GCN5*, real-time qRT-PCR was performed. Under the oxidative stress (H₂O₂), *SOD1* was downregulated in mutants except double mutants (*ada2 ada3* and *ada2 gcn5*) and *CTA1* was down-regulated in HAT mutants. However, *GPX2* was slightly upregulated in HAT mutants (Fig. 5). *C. glabrata* oxidative stress response is regulated by Yap1, Msn2, Msn4 and Skn7 (33). We further assessed the expression of transcription factors, *YAPI*, *MSN2*, *MSN4* and *SKN7*. Under the oxidative stress (H₂O₂), *YAPI*, *MSN2* and *MSN4* were not upregulated in HAT mutants (Data not shown); however HAT mutants increased the transcription of *SKN7* (Fig. 5). In addition, we found the complex lacking one of the genes (*ADA2*, *ADA3* or *GCN5*) enhanced the expression of other two HAT genes (Fig. 5), indicating compensation effect of the complex.

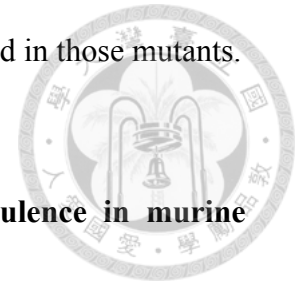
3.4 *C. glabrata* Ada3 and Gcn5 negatively regulate agar invasion and virulence associated genes

Adherence onto host surface is an important trait for colonization and infection. Deletion of *ADA2* in *C. glabrata* enhanced agar invasion (29) but whether Ada3 and



Gcn5 have conserved or divergent function in agar invasion is unknown. Deletion of *ADA3*, *GCN5*, *ADA2 ADA3*, *ADA2 GCN5 ADA3 GCN5* and *ADA2 ADA3 GCN5* showed robust agar invasion compared to the wild type and complementary strains (*ada3::ADA3* and *gcn5::GCN5*) (Fig. 6). Agar invasion is correlated to adhesion and it is mediated by Flo11 in *S. cerevisiae* (34, 35), whereas *C. glabrata* adherence to epithelial cells is mediated by Epa1 adhesin, which is a well-defined virulence factor (18). Loss of *GCN5* decreases virulence in *C. albicans* (21, 22), while *Aspergillus fumigatus* GcnE is not involved in virulence in murine models (23). Nevertheless, whether Ada3 and Gcn5 involve in regulation of virulence factors in *C. glabrata* remain elusive. Thus, we performed the real-time qRT-PCR to analyze the expression level of *EPA* genes. Under the oxidative stress, the expression level of *EPA1* were upregulated in *ada2* mutant, *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*) (Fig. 7). Two putative adhesins, *EPA20* and *EPA23* which are *ScFLO1* ortholog, were also upregulated in *ada2* mutant, *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*). In addition to adhesins, the other virulence factor, aspartyl proteases, encoded by *YPS* gene cluster are required for virulence and suppression of the host immune response (36, 37). Hence, we assessed the transcription level of *YPS* genes. *YPS1*, *YPS7* and *YPS (2-6 and 8-11)* are localized on different chromosome. Here we chose *YPS1* and *YPS7* as these genes are crucial in disseminated infection and chose *YPS4* and *YPS10* since these genes are induced by macrophages (37). Under oxidative stress, the transcription of *YPS7*, *YPS4* and *YPS10* were upregulated in *ada2* mutant, *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada3 gcn5*) and

triple mutant (*ada2 ada3 gcn5*); however, *YPS1* was not upregulated in those mutants.



3.5 Deletion of both *ADA3* and *GCN5* resulted in hypervirulence in murine systemic infection model

In previous study, deletion of *ADA2* increased virulence in murine systemic infection model (29). Based on the fact that deletion of *ADA3* or *GCN5* enhanced agar invasion and transcription of virulence associated genes, we hypothesize that *ada3* or *gcn5* mutant will exhibit hypervirulence similar to *ada2* mutant in murine systemic infection model. Interestingly, we found the deletion of *ADA3* or *GCN5* resulted in marginal hypervirulence (WT vs. *ada3*, $P = 0.1707$; WT vs. *gcn5*, $P = 0.0566$), while deletion of both *ADA3* and *GCN5* resulted in hypervirulence (WT vs. *ada3 gcn5*, $P = 0.0038$) in murine systemic infection model (Fig. 8). Meanwhile, *ada2 ada3*, *ada2 gcn5* and *ada2 ada3 gcn5* exhibited hypervirulence (Fig. 8), indicating a critical role of *ADA2* in negatively regulate virulence. The virulence was similar between the wild type and complementary strains (WT vs. *ada3::ADA3*, $P = 0.3495$; WT vs. *gcn5::GCN5*, $P = 0.9536$).



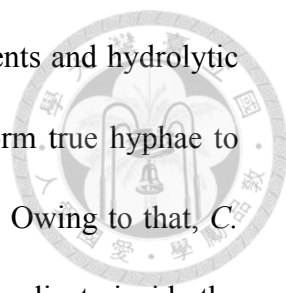
4. Discussion

4.1 The roles of *C. glabrata* Ada3 and Gcn5 in growth

SAGA complex is a general cofactor in transcription by recruiting the RNA polymerase II and general transcription factors that regulates global gene expressions through histone post modification (38, 39). Previous studies show that *ADA2*, *ADA3* and *GCN5* play a role in response to cell cycle progression, while *GCN5* regulates the respiratory in *S. cerevisiae* (14, 15, 40). Deletion of *ADA2* in *C. albicans* results in a mild growth defect while *C. glabrata* without *ADA2* decreased growth rate at the elevated temperatures (29, 41). However, whether deletion of *ADA3* or *GCN5* have a convergent or divergent role in growth in *C. glabrata* is still unknown. In this study, deletion of *ADA3* and *GCN5* exhibited a slower growth rate after 9 h. Strains lack of *ADA2 ADA3*, *ADA2 GCN5*, *ADA3 GCN5* and *ADA2 ADA3 GCN5* grew similar to single mutants (*ada2*, *ada3* or *gcn5*), indicating that there is no dominant role in growth among *Ada2*, *Ada3* and *Gcn5*, provided the evidence that *ADA2*, *ADA3* and *GCN5* regulate growth within a similar pathway (32, 42). *Gcn5* plays a role in DNA replication rate (16) and deletion of *ADA2* and *ADA3* in *S. cerevisiae* reduces *CLN3* expression which regulates the cell progression (14). This suggests that deletion of *ADA3* and *GCN5* resulted in a longer S phase and G₁ phase that delay the cell progression in *C. glabrata*.

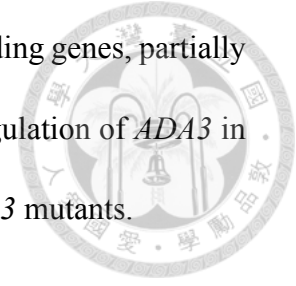
4.2 The roles of *C. glabrata* Ada3 and Gcn5 in stress responses

In the initiation of *Candida* infection, the first line of immune system, phagosomes (macrophages and neutrophils), will be attracted and engulf *Candida*, and uses ROS



generated by NADPH oxidase, low pH value, deprivation of nutrients and hydrolytic activity to kill *Candida* (43-45). However, *C. glabrata* can not form true hyphae to escape from phagosomes as *C. albicans* does for immune evasion. Owing to that, *C. glabrata* has developed an unusual stress response to survive and replicate inside the phagosomes, behaves like Trojan horse (44, 46). *CgADA2* is critical to against oxidative stress in *Drosophila* larvae model (24) while *CaADA2* involves in gene regulation which is recruited to about 200 promoters related to stress response including oxidative stress (47). Our results revealed that loss of *ADA3* or *GCN5* in *C. glabrata* increased susceptibility to ROS and cell wall perturbing agents, indicating *ADA3* and *GCN5* have conserved function similar to *ADA2*. Interestingly, *C. glabrata* without *GCN5* grew intermediate under ROS (i.e., H₂O₂ and menadione) compared to the wild type and *ada2* or *ada3* mutant. Furthermore, the expression of oxidative stress response genes, *SOD1* and *CTA1*, were down-regulated in *ada2* mutant, *ada3* mutant, *gcn5* mutant, double mutant (*ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*) when cells were grown with H₂O₂. *YAP1*, *MSN2*, *MSN4* and *SKN7* are involved in *CTA1* regulation in *C. glabrata* (48). We found that *YAP1*, *MSN2* and *MSN4* were not regulated in HAT mutants while *SKN7* was slightly upregulated. The regulation of oxidative response genes (i.e., *CTA1*) depends on co-operate by Yap1 and Skn7 in *S. cerevisiae* (49, 50), suggesting that HAT mutants can not respond to ROS without the upregulation of *YAP1* although the expression of *SKN7* was subtle increased. Deletion of *ADA3* and *GCN5* increased susceptibility to oxidative stress and cell wall perturbing agents may due to several dimensions, not only the lower transcription of antioxidative stress response gene but also defect the fitness of growth. Besides, the compensation effect that deletion of

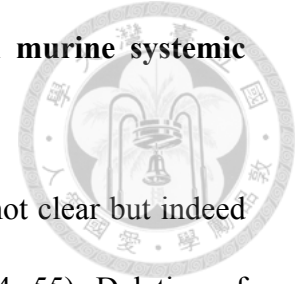
GCN5 in *Fusarium fujikuroi* increased the expression of HAT-encoding genes, partially explain the upregulation of *ADA2* in *ada3* and *gcn5* mutants, upregulation of *ADA3* in *ada2* and *gcn5* mutants and upregulation of *GCN5* in *ada2* and *ada3* mutants.



4.3 The roles of *C. glabrata* Ada3 and Gcn5 in drug tolerance

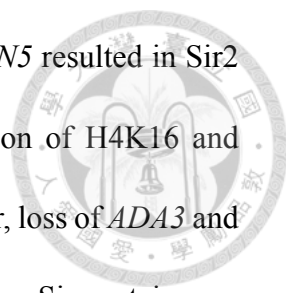
Antifungal drug resistance is being a large concern in recent years. The emerging drug resistance strains is an inevitable consequence of constraining treatment. Deletion of *ADA2* in *C. glabrata* decreased antifungal drug tolerance (29). Whether *C. glabrata* *ADA3* and *GCN5* have conserved or divergent function in antifungal drug tolerance remain elusive. *ada3* mutant, *gcn5* mutant, double mutants (*ada2 ada3*, *ada2 gcn5* and *ada2 ada3 gcn5*) and triple mutant (*ada2 ada3 gcn5*) decreased tolerance to FLC, PSC, VRC, MCF, CSF, ANF and AmB. The MIC results indicated that Ada2, Ada3 and Gcn5 play a similar role to against antifungal drug. The histone acetyltransferase activity is required for antifungal drug tolerance or resistance. For example, disruption of *ADA3* in *S. cerevisiae* decreases tolerance to FLC (51) while loss of *GCN5* in *C. albicans* or *Cryptococcus neoformans* increases susceptibility to caspofungin (21, 41) or FK506 (20), respectively. Multiple efflux pump Mdr1 is crucial in drug resistance. Previous studies have indicated that Ada2 and Gcn5 are involved the regulation of *MDR1* in *C. albicans* (21, 52). Interestingly, gain of function mutation in *CgPDR1* is common in clinical with an increased antifungal drug tolerance. However it is synthetically lethal in the absence of *GCN5* (53), implied that target of Gcn5 or even Ada3 and Ada2 can reduce the emergence of antifungal drug tolerance.

4.4 The roles of *C. glabrata* Ada3 and Gcn5 in virulence in murine systemic infection model



The relationship between the agar invasion and virulence is not clear but indeed provides a high throughput screen to determine the virulence (54, 55). Deletion of *ADA3*, *GCN5*, *ADA2 ADA3*, *ADA2 GCN5*, *ADA3 GCN5* and *ADA2 ADA3 GCN5* increased agar invasion which is similar to *ada2* mutant. Whereas in fission yeast, *Schizosaccharomyces pombe*, deletion of *GCN5* reduced invasive growth (56), suggesting the divergent role in invasive growth between *S. pombe* and *C. glabrata*. In addition, several studies have indicated that SAGA complex involves in virulence in yeasts and filamentous fungi (19-22, 57, 58). For example, deletion of *ADA2*, *ADA3* or *GCN5* in *C. albicans* attenuates virulence in *Caenorhabditis elegans* infection model and murine systemic infection model, respectively (21, 22, 58, 59), while deletion of *ADA2* in *C. glabrata* resulting in hypovirulence in *Drosophila* model (24) or hypervirulence in murine systemic infection model (29). Nevertheless, despite defect in growth, cell wall integrity and oxidative stress response, deletion of *ADA3* or *GCN5* increased transcription level of virulence associated genes (i.e., *EPA1*, *EPA20*, *EPA23*, *YPS4*, *YPS7* and *YPS10*) and resulted in a marginal hypervirulence, but deletion of both *ADA3* and *GCN5* resulted in hypervirulence in murine systemic infection model. The robust invasive growth and increased expression of virulence associated genes of HAT mutants might partially explain the hypervirulence.

Sir proteins including Sir2, Sir3 and Sir4 are NAD⁺-dependent histone deacetylase which involved in subtelomeric silencing. Disruption of *SIR3* and *RIFI* increases the transcription of *C. glabrata* *EPA1*, *EPA6* and *EPA7* due to the defect of subtelomeric



silencing (60, 61). In addition, *S. cerevisiae* without *ADA2* and *GCN5* resulted in Sir2 and Sir3 moving to subtelomeric DNA resulting in hypoacetylation of H4K16 and elevation of interaction between histone and DNA (62, 63). However, loss of *ADA3* and *GCN5* in *C. glabrata* increased the transcription of *EPA1*, indicating Sir proteins are not moving to subtelomeric regions and suppressing gene expressions. This suggests the divergent function of HAT module between *C. glabrata* and *S. cerevisiae* that components of HAT module might not involve in SIR-dependent telomere silencing. Besides the regulation of adhesins, three yapsins (*YPS4*, *YPS7* and *YPS10*) were upregulated in HAT mutants. There are two possibilities that deletion of *ADA3* and *GCN5* increased the transcription of *YPS* genes. One is *YPS* genes are regulated by Ada3 and Gcn5 negatively and the other one is deletion of *ADA3* and *GCN5* decreased the cell wall integrity which might be a signal for compensation effect since *YPS* family genes are involved in cell wall metabolism and induced by various environmental cues. For example, *C. glabrata YPS1* is induced by SDS and growth at 37°C (64). *C. glabrata YPS4*, *YPS5*, *YPS8* and *YPS10* play a crucial role in intracellular survival (36), indicating that deletion of *ADA3* and *GCN5* might enhance intracellular survival and virulence in murine systemic infection model.

5. Tables

Table 1. *Candida* strains used in this study

Strain	Genotype	Parent	Reference
<i>C. glabrata</i> (CBS138)	Prototrophic wild type	Clinical isolate	(26)
YSJ39	<i>ada2Δ::SAT1-FLP</i>	CBS138	(25)
YSJ43	<i>ada2Δ::FRT</i>	YSJ39	(25)
YSJ68	<i>ada3Δ::SAT1-FLP</i>	CBS138	This study
YSJ115	<i>ada3Δ::FRT</i>	YSJ68	This study
YSJ126	<i>ada3Δ::FRT::ADA3-SAT1-FLP</i>	YSJ115	This study
YSJ65	<i>gcn5Δ::SAT1-FLP</i>	CBS138	This study
YSJ104	<i>gcn5Δ::FRT</i>	YSJ65	This study
YSJ108	<i>gcn5Δ::FRT::GCN5-SAT1-FLP</i>	YSJ115	This study
YSJ74	<i>ada2Δ::FRT ada3Δ::SAT1-FLP</i>	YSJ43	This study
SY26	<i>ada2::FRT gcn5Δ::SAT1-FLP</i>	YSJ43	This study
YSJ131	<i>ada2Δ::FRT ada3Δ::FRT</i>	YSJ74	This study
SY20	<i>ada3Δ::FRT gcn5Δ::SAT1-FLP</i>	YSJ115	This study
SY16	<i>ada2Δ::FRT ada3::FRT gcn5::SAT1-FLP</i>	YSJ131	This study
<i>C. krusei</i> (CBS573)	Prototrophic wild type	Clinical isolate	(65)

Table 2. Plasmids used in this study

Plasmid	Relevant insert	Parent
pSFS2A		(27)
pYSJ59	3' <i>GCN5</i> ^{NCR}	pSFS2A
pYSJ61	5' <i>GCN5</i> ^{NCR}	pYSJ59
pYSJ101	5' <i>GCN5</i> ^{NCR} – <i>GCN5</i>	pYSJ59
pYSJ58	3' <i>ADA3</i> ^{NCR}	pSFS2A
pYSJ63	5' <i>ADA3</i> ^{NCR}	pYSJ58
pYSJ103	5' <i>ADA3</i> ^{NCR} – <i>ADA3</i>	pYSJ58





Table 3. PCR primers used in this study

Primer	Use	Sequence (5' to 3')
JC717	3' <i>GCN5</i> ^{NCR}	AAACCGCGGCTATTCTTCTGAGTTTGTAAC
JC718	3' <i>GCN5</i> ^{NCR}	AAAGAGCTCTGGGACTTTAGAGGCTTTTG
JC715	5' <i>GCN5</i> ^{NCR}	AAAGGTACCGCATAATTCATACAAACG
JC716	5' <i>GCN5</i> ^{NCR}	AAAGGGCCCCCTTTGTTGTTTCTTGCTAGT
JC639	<i>GCN5</i> ORF	TACGAGGAGGAGATTGCATCA
JC640	<i>GCN5</i> ORF	AGACCAGTCAAGACCATCATA
JC48	5' <i>SAT1-FLP</i>	ACAATCAAAGGTGGTCCT
JC81	3' <i>SAT1-FLP</i>	AACTTCCTCGAGGGGGGGCC
JC1076	5' integration (<i>gcn5</i>)	AACCATTGAGAAGTTGCCTTG
JC1077	3' integration (<i>gcn5</i>)	ATCCAGGAACGGATGATGAGT
JC1307	<i>GCN5</i> complement	AAACTCGAGGGTTTTTCATGAATAAATGAGG
JC1308	<i>GCN5</i> complement	AAAAAGCTTCTAATCGATCAAATGTGAGTA
JC906	3' <i>ADA3</i> ^{NCR}	AAAGCGGCCCGCCACAGCCTACATAAGCACCAA
JC907	3' <i>ADA3</i> ^{NCR}	AAAGAGCTCACTCGAACTCCTTGACGATCT
JC904	5' <i>ADA3</i> ^{NCR}	AAAGGGCCCTAGGGTGCTCTTCACCCACAT
JC905	5' <i>ADA3</i> ^{NCR}	AAACTCGAGTCTTTAGCCCATTCACCACG
JC908	<i>ADA3</i> ORF	GGAAGAAAACGAAGGCACAGA
JC909	<i>ADA3</i> ORF	GCATTGTCATTATCCTTTGAG
JC910	5' integration (<i>ada3</i>)	TTGCAGTAGCCTCTTCAGGAT
JC911	3' integration (<i>ada3</i>)	ATAATGGGCGTGTTAGGTGA
JC1309	<i>ADA3</i> complement	AAACTCGAGCTAGTCTTCCAGTCCTTCTTC
JC873	qPCR <i>ADA2</i> ORF	GCCTCCGTGCCGTCTTG
JC874	qPCR <i>ADA2</i> ORF	CCAAAGGTTGATCATCTGGTTCA
JC1889	qPCR <i>ADA3</i> ORF	TGCGGATCAAAGAGGAGGAA
JC1890	qPCR <i>ADA3</i> ORF	GCCTCGGGACTTTCAGCTTT
JC1891	qPCR <i>GCN5</i> ORF	GAACAATCCACCGAGGACCA
JC1892	qPCR <i>GCN5</i> ORF	TCCGTGCCTTCATCATTCTG
JC1933	qPCR <i>EPA1</i> ORF	CCATCTGGGGCTCAAAAACA
JC1934	qPCR <i>EPA1</i> ORF	GCAGCCCTCCTCTGTGTCAT

Table 3. continued

Primer	Use	Sequence (5' to 3')
JC1046	qPCR <i>EPA20</i> ORF	TGTC AAGCCATCCAGTTCAGTT
JC1047	qPCR <i>EPA20</i> ORF	TAACCGTCTGTACATATCGTTGCA
JC1042	qPCR <i>EPA23</i> ORF	TGATACTTCCCCC AAAACG
JC1043	qPCR <i>EPA23</i> ORF	TGGTTC ACTTGATATGGCTGATG
JC1994	qPCR <i>YPS1</i> ORF	TGAGCTAAAGAGGGGCGCTTG
JC1995	qPCR <i>YPS1</i> ORF	AGTTGGCTGAGCGGAGTCTG
JC1899	qPCR <i>YPS4</i> ORF	GTGGTCCATGGGGAGTTGAT
JC1900	qPCR <i>YPS4</i> ORF	TTCTAGAGCCATTGGCAGCA
JC1996	qPCR <i>YPS7</i> ORF	ACGGATTTCGCAGACACCAGT
JC1997	qPCR <i>YPS7</i> ORF	CCCATAGGCACCACAGGGTA
JC1048	qPCR <i>YPS10</i> ORF	GGACACGGGTTCGTCTGATT
JC1049	qPCR <i>YPS10</i> ORF	TGCATCTAAGGAGGCAATGCT
JC1875	qPCR <i>SOD1</i> ORF	CCTCCGAACAGGACCCTACC
JC1876	qPCR <i>SOD1</i> ORF	CACCGACGTGTCTGTTCTCG
JC1877	qPCR <i>CTA1</i> ORF	AAGGTCTGGCCACACAAGGA
JC1878	qPCR <i>CTA1</i> ORF	GAGGCGTATGGGCAGTTGAC
JC1879	qPCR <i>GPX2</i> ORF	AGGGCTTGGTCATCCTTGGT
JC1880	qPCR <i>GPX2</i> ORF	ACCCAGCAGGCCACTCTTTT
JC1881	qPCR <i>SKN7</i> ORF	CCGGTGACAGTTTTGTGGTG
JC1882	qPCR <i>SKN7</i> ORF	CGAACTCCCAGCTTTGTTCG
JC1901	qPCR <i>CAGL0M07634g</i> ORF	GCACGAAGCTGCTGAATGTC
JC1902	qPCR <i>CAGL0M07634g</i> ORF	GCCATTGCTCTATGCCTTCC
JC853	qPCR <i>CAGL0L01771g</i> ORF	CAGTCGCTGGAGATGGTAAGG
JC854	qPCR <i>CAGL0L01771g</i> ORF	ACCACCGACACGCCATTAG

1. Restriction enzyme enzyme cutting site are underline.

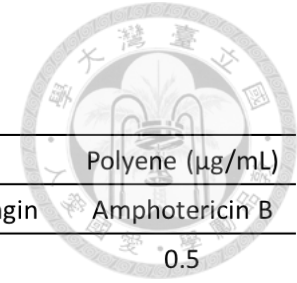


Table 4. The minimal inhibitory concentrations of antifungal drug against *C. glabrata* strains

	Azoles (µg/mL)			Echinocandins (µg/mL)			Polyene (µg/mL)
	Fluconazole	Posaconazole	Voriconazole	Micafungin	Caspofungin	Anidulafungin	Amphotericin B
<i>C. glabrata</i> (CBS138)	8	0.5	0.125	0.06	0.25	0.125	0.5
<i>ada2</i>	2	0.125	0.03	0.008	0.25	0.06	0.125
<i>ada3</i>	2	0.125	0.015	0.008	0.5	0.125	0.25
<i>ada3::ADA3</i>	8	0.25	0.125	0.06	0.5	0.125	0.5
<i>gcn5</i>	2	0.25	0.03	0.015	0.25	0.06	0.25
<i>gcn5::GCN5</i>	8	0.25	0.125	0.03	0.25	0.125	0.5
<i>ada2 ada3</i>	2	0.125	0.03	0.008	0.25	0.125	0.25
<i>ada2 gcn5</i>	2	0.06	0.03	≤0.008	0.25	0.06	0.125
<i>ada3 gcn5</i>	2	0.125	0.03	0.008	0.25	0.06	0.125
<i>ada2 ada3 gcn5</i>	2	0.125	0.06	0.008	0.25	0.06	0.125
<i>C. krusei</i> (CBS573)	32	0.125	0.25	0.25	1	0.125	1

C. krusei as reference

6. Figures

Figure 1

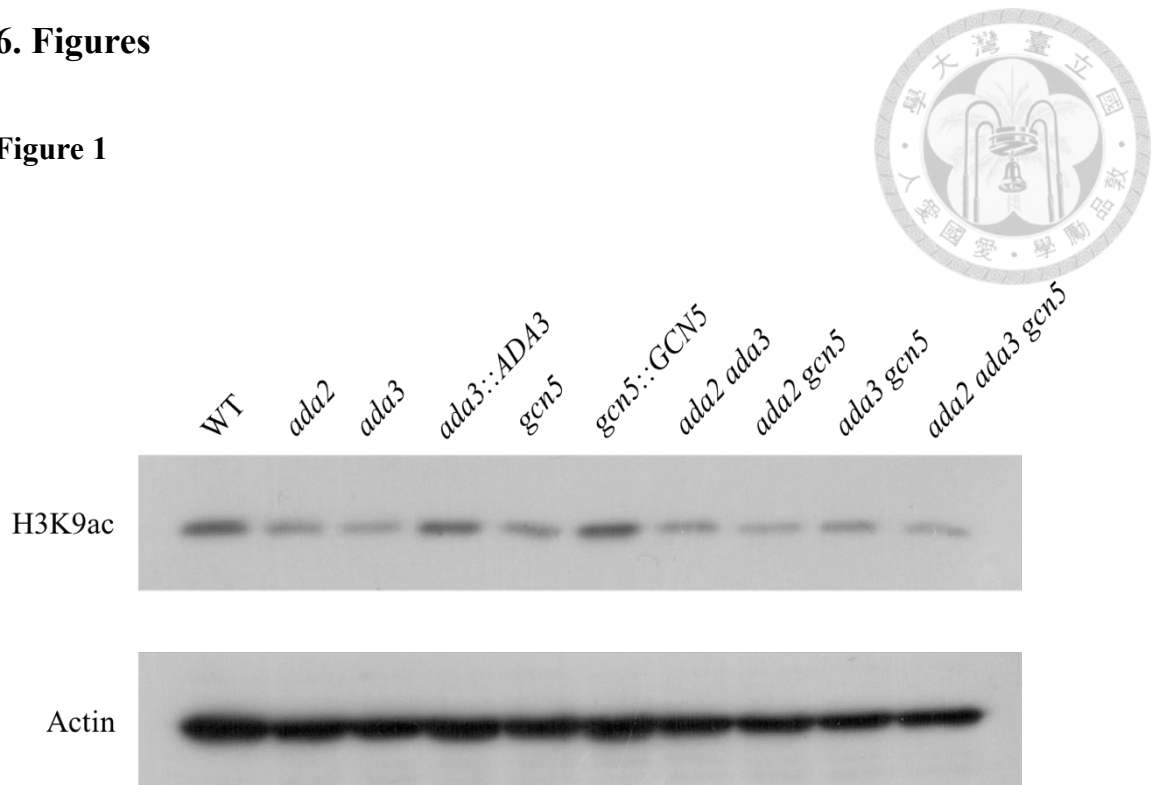


Figure 1. Deletion of *ADA3* and *GCN5* decreased the acetylation level of H3K9 in *C. glabrata*.

C. glabrata strains were grown at 37°C and total proteins were extracted. 10 µg of crude extracted proteins were separated by protein electrophoresis and transferred onto nitrocellulose, then probed with anti-H3K9ac antibody to determine the acetylation level. The anti-actin antibody was used as an internal control. Three biological repeats were conducted and the bolts were analyzed using ImageJ software. Asterisks indicate statistically significant difference compared to the wild type using one way ANOVA (* $P < 0.05$).

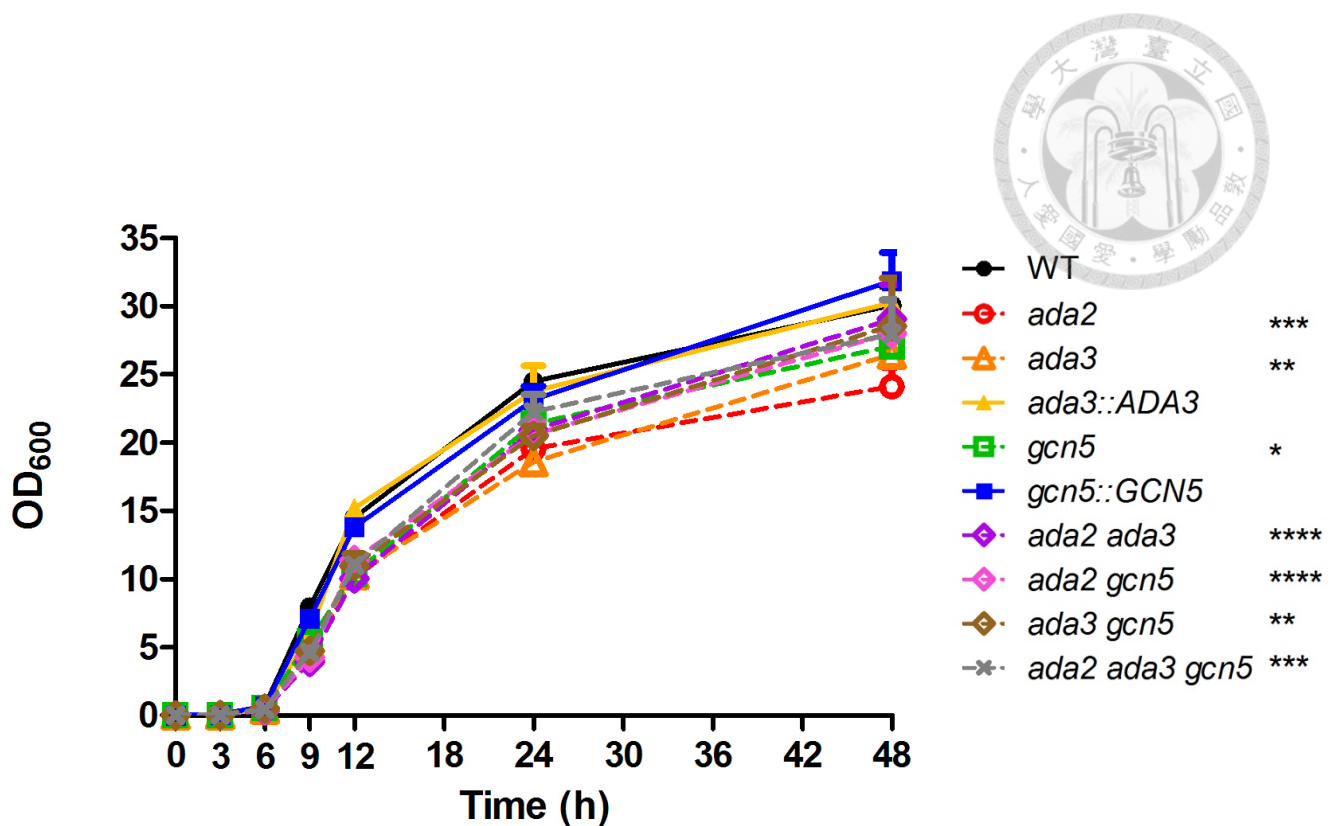


Figure 2. *C. glabrata* Ada3 and Gcn5 were contributed to growth at 37°C.

C. glabrata strains were grown overnight at 30°C, washed twice with ddH₂O and then diluted to 0.01 OD₆₀₀ with fresh YPD broth. Measurements were conducted at 0, 3, 6, 9, 12, 24 and 48 h after incubation at 37°C using SpectraMax 190 microplate reader. Plot was drawn using Prism software (v5.03). The error bars represent standard deviations from three technical repeats. Asterisks indicate statistically significant difference compared to the wild type using two way ANOVA (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$).

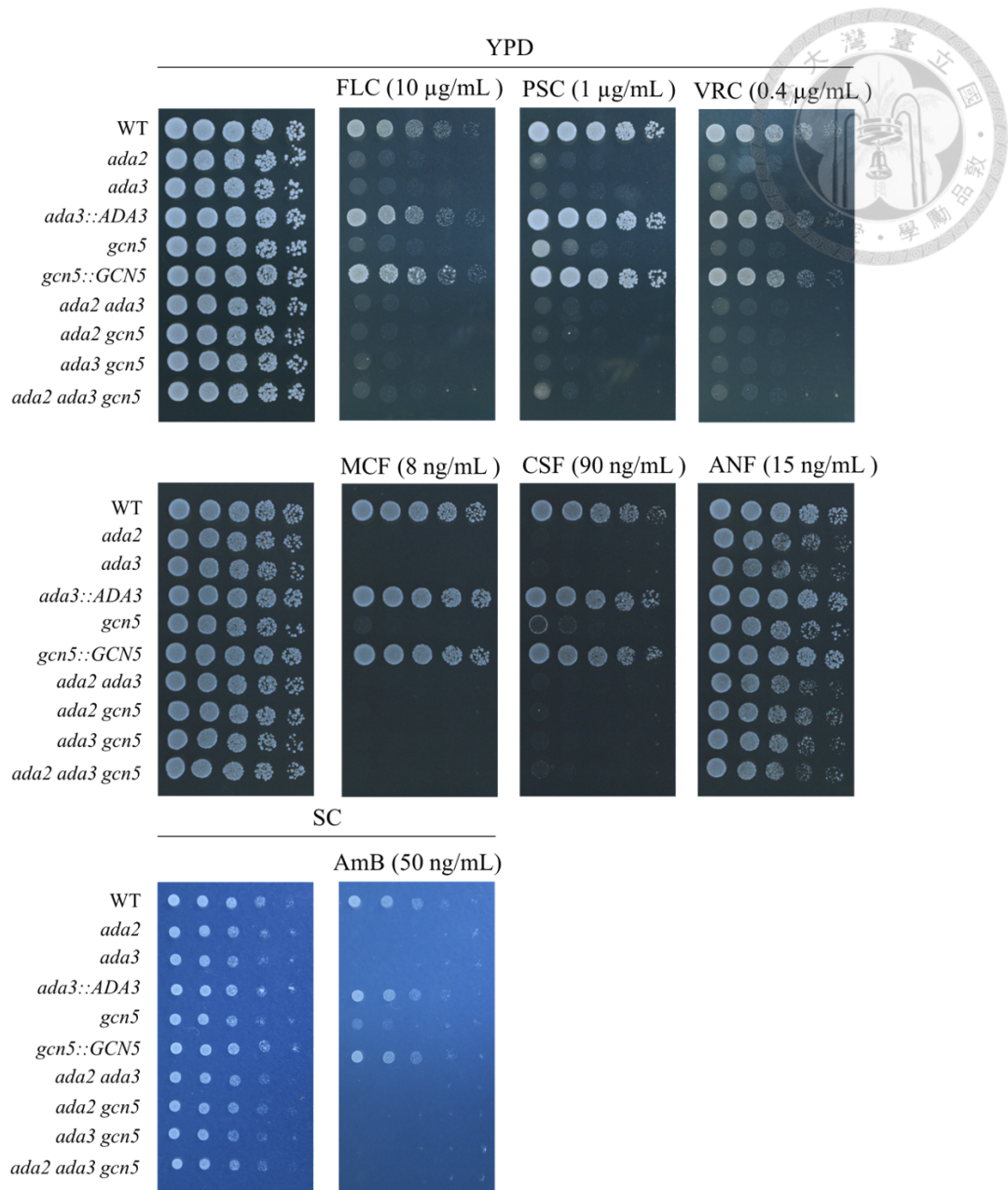


Figure 3. *C. glabrata* Ada3 and Gcn5 were involved in drug tolerance.

C. glabrata strains were grown overnight at 30°C with shaking at 200 rpm, washed twice with ddH₂O, diluted to 1 OD₆₀₀ with ddH₂O, and then 3 µl of 5-fold serial dilution of cell suspensions were spotted onto YPD plate in the absence or presence of antifungal drug. The plates were incubated at 37°C for 24 h and photographed. Fluconazole (FLC); Posaconazole (PSC); Voriconazole (VRC); Miconazole (MCF); Caspofungin (CSF); Anidulafungin (ANF); Amphotericin B (AmB).

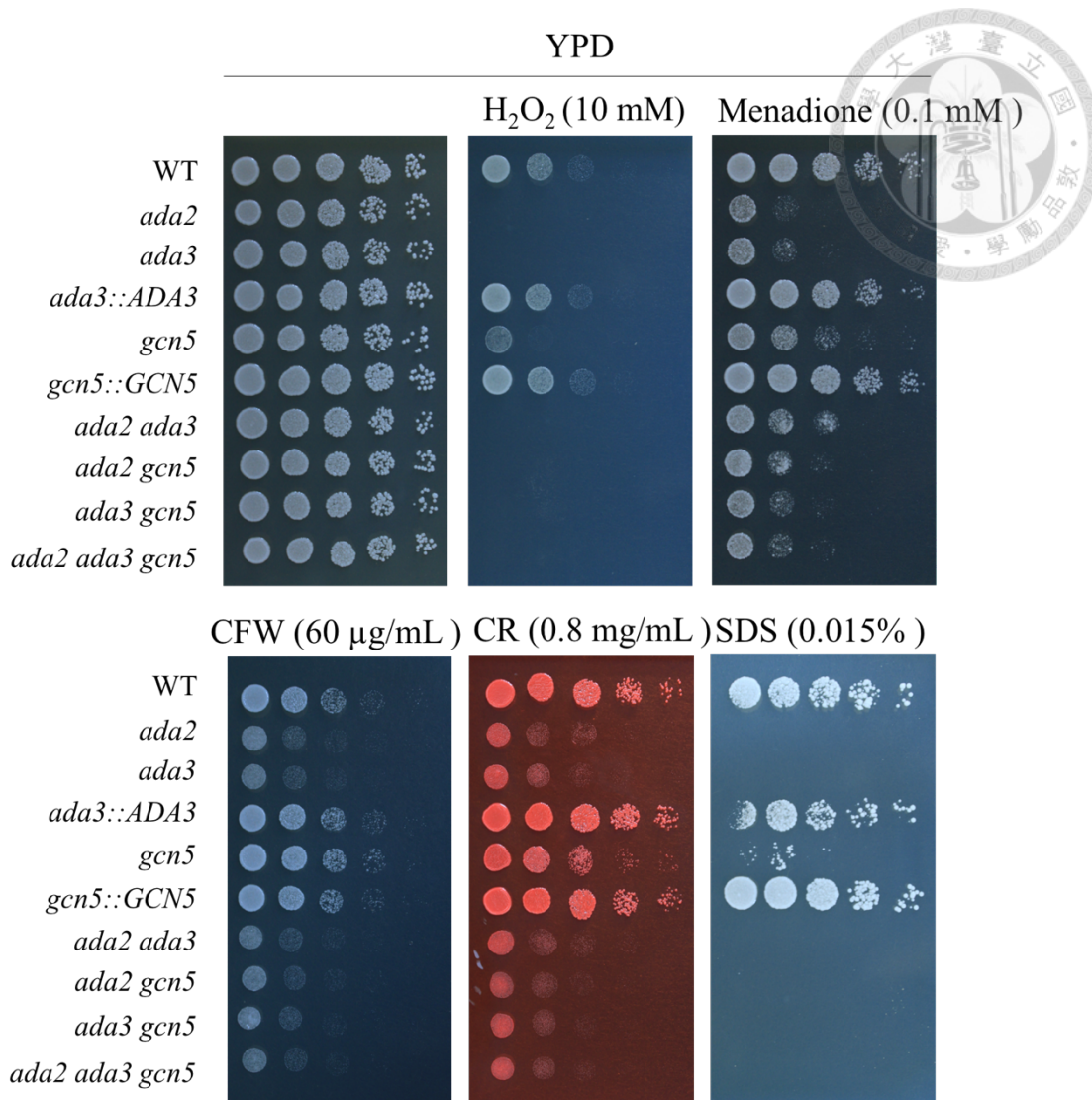


Figure 4. *C. glabrata* Ada3 and Gcn5 were crucial in oxidative response and cell wall integrity.

C. glabrata strains were grown overnight at 30°C with shaking at 200 rpm, washed twice with ddH₂O, diluted to 1 OD₆₀₀ with ddH₂O, and then 3 µl of 5-fold serial dilution of cell suspensions were spotted onto YPD plate in the absence or presence of reactive oxygen species (H₂O₂ or menadione) or cell wall perturbing agents (CFW, CR or SDS). The plates were incubated at 37°C for 24 h except SDS plate for 48 h and photographed. Hydrogen peroxide (H₂O₂); Menadione ; Calcofluor white (CFW); Congo red (CR) Sodium dodecyl sulfate (SDS).

Figure 5

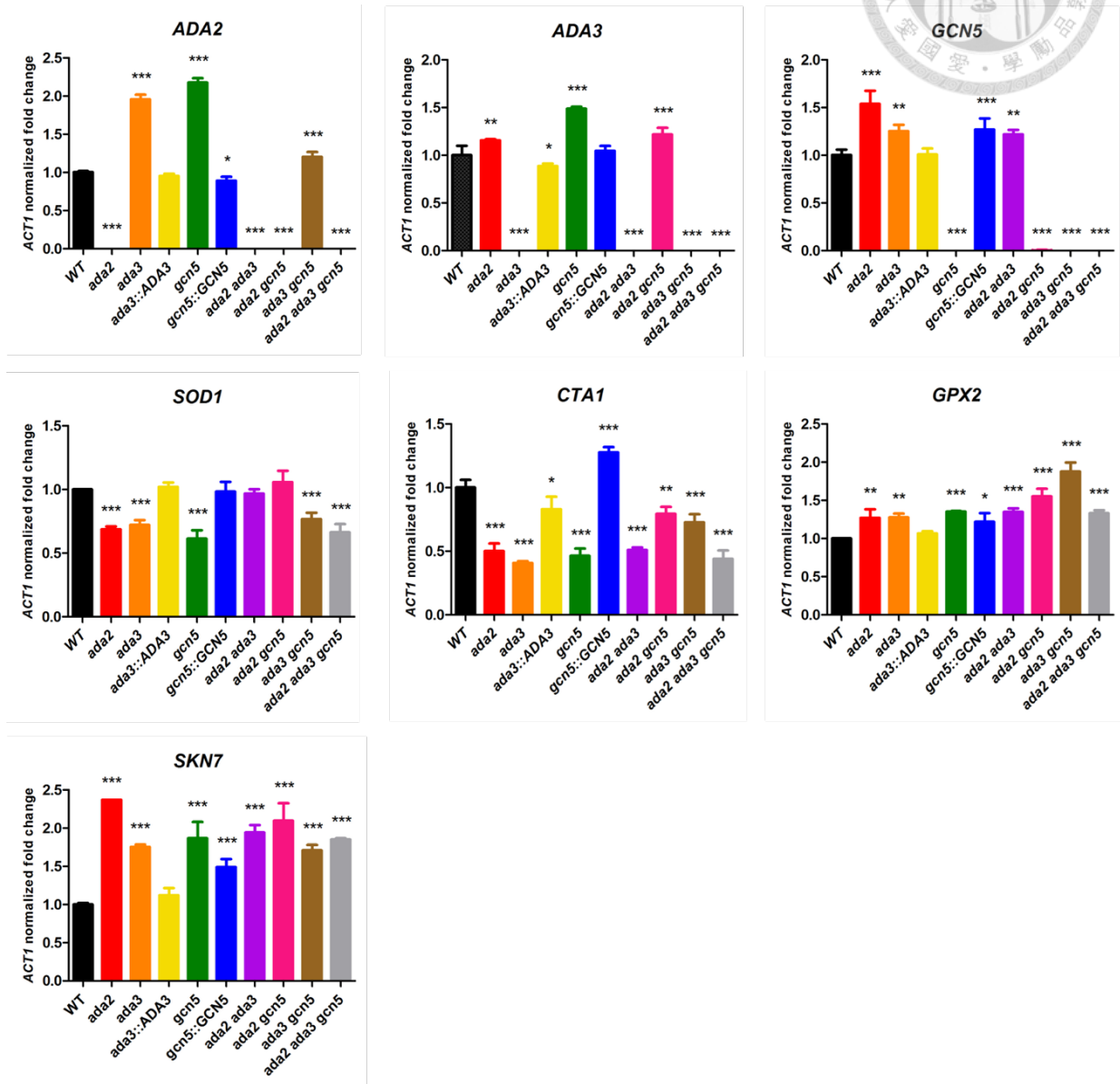


Figure 5. *C. glabrata* Ada3 and Gcn5 were required for oxidative stress response by regulating *SOD1* and *CTA1*.

C. glabrata strains were grown overnight in YPD broth at 30°C, adjusted to 0.2 OD₆₀₀/mL with fresh YPD broth with 2 mM H₂O₂, and incubated at 37°C for 3 h. Total RNA was extracted using TRIzol and Turbo DNA-free kit to eliminate DNA, then 1 µg DNA-free RNA was reverse transcribed to cDNA using high capacity reverse transcription kit. Transcription levels were analyzed by SYBR[®] Green PCR Master Mix. StepOnePlus system and StepOne (v2.2) system were used to determine cycle threshold (C_T) value. *C. glabrata* *ACT1* expression was used to normalize the relative quantity for comparing with wild type. *P* values were determined using one way ANOVA. (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

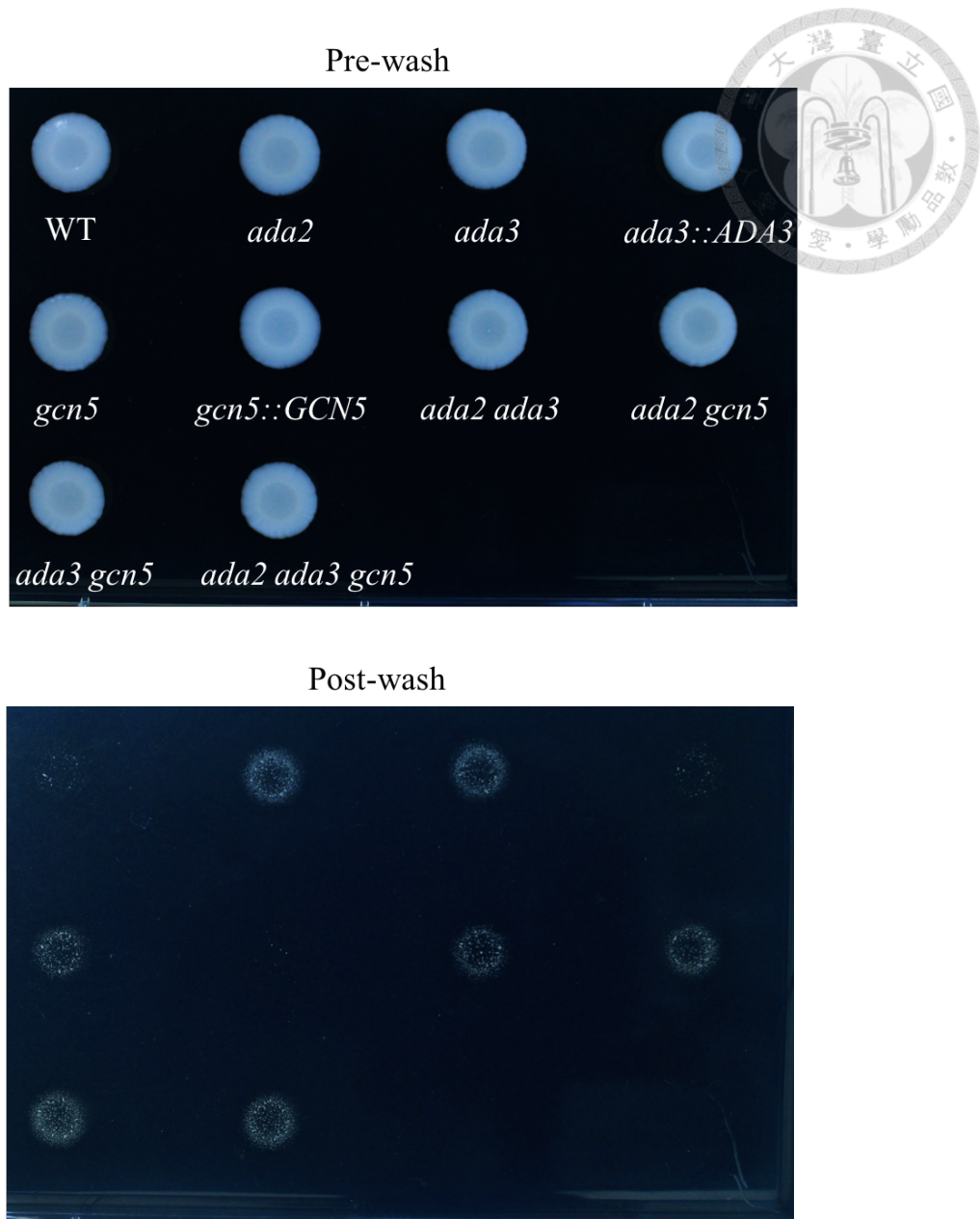


Figure 6. Deletion of *C. glabrata* *ADA3* or *GCN5* enhanced agar invasion.

C. glabrata strains were grown overnight at 30°C with shaking at 200 rpm, washed twice with ddH₂O. Cell suspensions were diluted to 1 OD₆₀₀ and 3 μL were spotted on YPD, incubated at 37°C for 3 days, then colonies were removed using swab and washed with dH₂O.

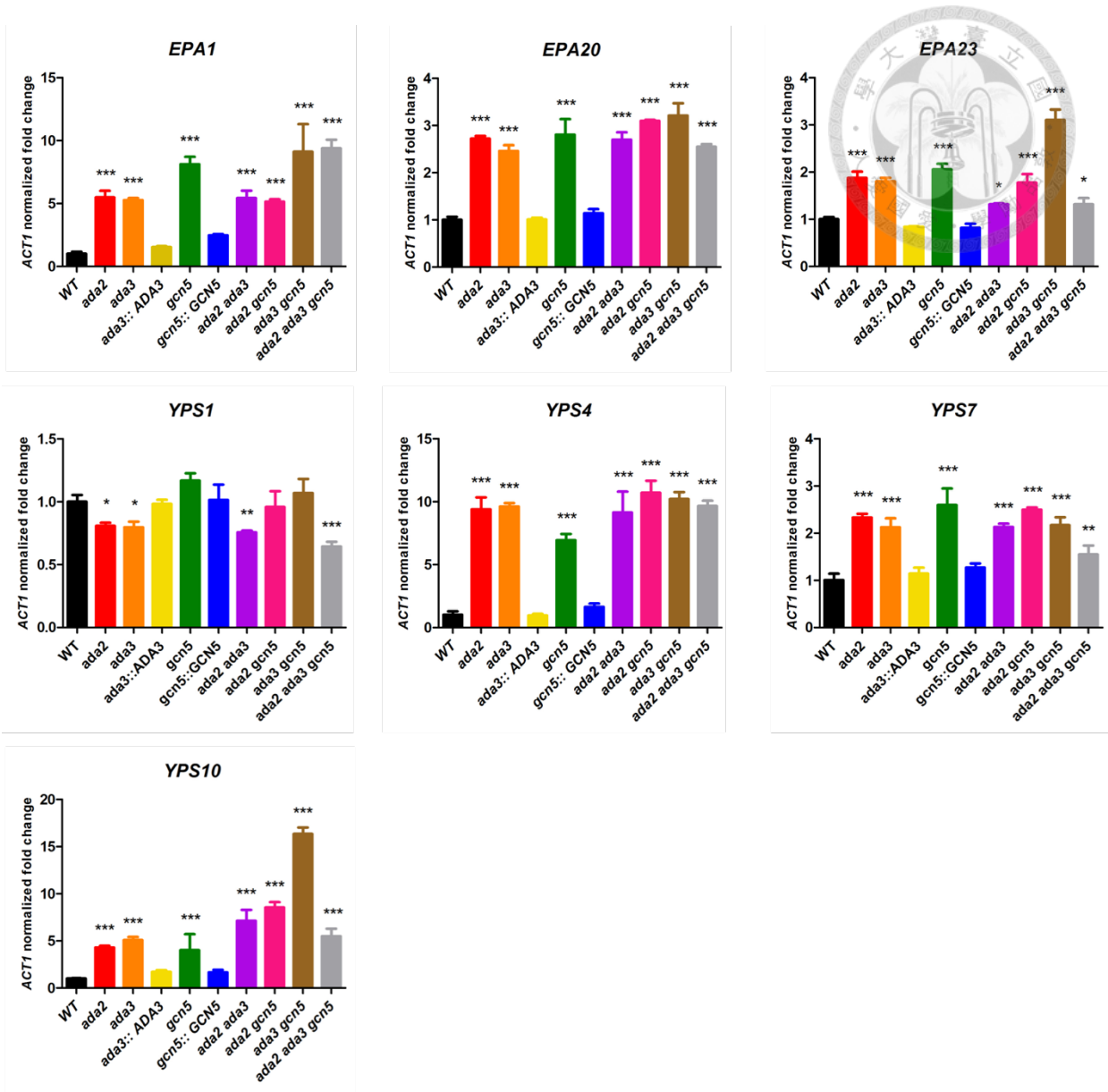
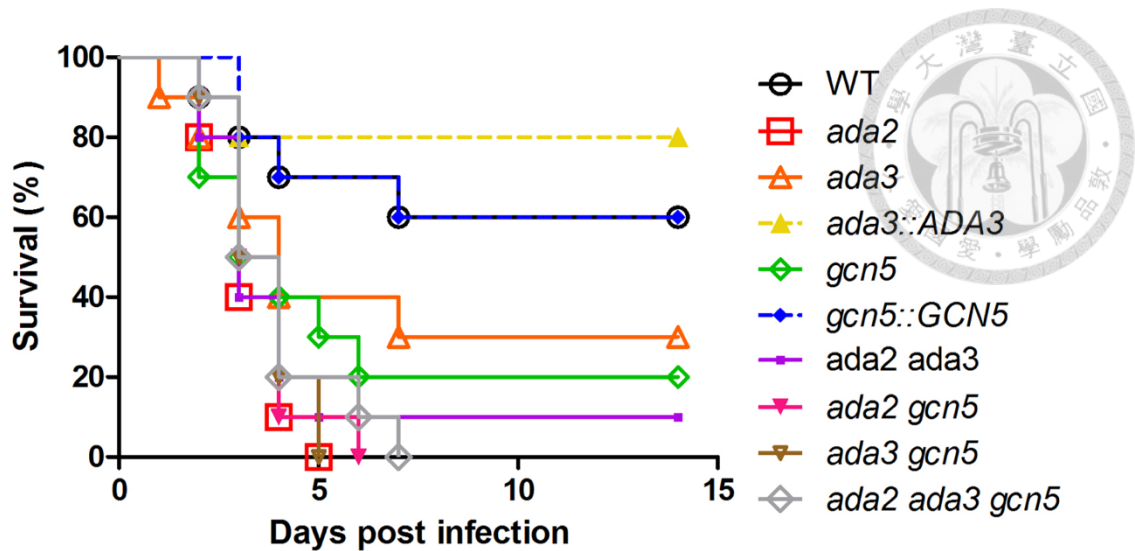


Figure 7. Defect of HAT module increased expression of most virulence associated genes.

C. glabrata strains were incubated in fresh YPD broth with 2mM H₂O₂ at 37°C. Total RNA was extracted and reverse transcribed into cDNA. Two virulence related gene family, *EPA* gene family and *YPS* gene family, were upregulated in HAT mutants.



	Curve comparison	<i>P</i> value	Significance
WT	<i>ada2</i>	0.0023	**
WT	<i>ada3</i>	0.1707	NS
WT	<i>ada3::ADA3</i>	0.3495	NS
WT	<i>gcn5</i>	0.0566	NS
WT	<i>gcn5::GCN5</i>	0.9536	NS
WT	<i>ada2 ada3</i>	0.0148	*
WT	<i>ada2 gcn5</i>	0.0035	**
WT	<i>ada3 gcn5</i>	0.0038	**
WT	<i>ada2 ada3 gcn5</i>	0.0045	**

Figure 8. Deletion of both *ADA3* and *GCN5* exhibited hypervirulence in murine systemic infection model.

Five to six-week-old male ICR mice were used in this study. 10 mice as one group and all groups were administrated with 150 mg/kg cyclophosphamide at day -3, 0, 1. *C. glabrata* strains were grown in liquid YPD overnight at 30°C, washed twice with phosphate-buffered saline then adjusted to 3.5×10^8 cells/mL. 200 μ L (7×10^7 cells) cell suspension was used for lateral tail vein injection and mice were monitored for 14 days. Statistical analysis was conducted using Mantel-Cox log-rank test.

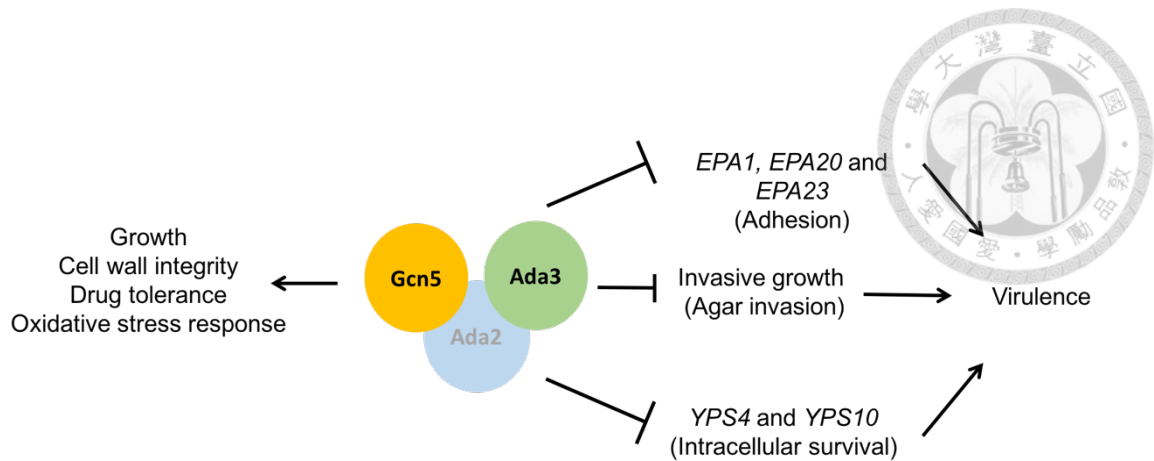


Figure 9. Proposed roles of HAT module within SAGA complex in drug tolerance and virulence in *C. glabrata*.

C. glabrata Ada3 and Gcn5 have conserved roles in oxidative stress response, cell wall integrity and drug tolerance. Interestingly, loss of *GCN5* in *C. glabrata* have an intermediated response to oxidative stress, cell wall perturbing agents and drug tolerance (i.e., FLC, PSC, MCF). In addition, we found *ADA3* and *GCN5* might play as negative regulators in virulence that deletion of *ADA3* and *GCN5* enhance agar invasion, upregulate transcription of adhesion associated genes (*EPA1*, *EPA20* and *EPA23*) and intracellular survival associated genes (*YPS4* and *YPS10*), leading to hypervirulence in murine systemic infection model.

7. Supplementary

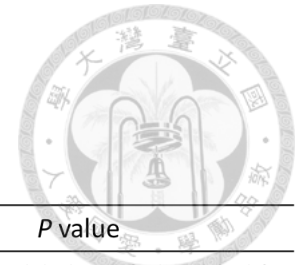


Table S1. Doubling time of the *C. glabrata* strains at 37°C

Strain	Doubling time (min) at 37°C	P value
<i>C. glabrata</i> (CBS138)	45.18 ± 1.99	A1, A2, A3, A4, A5, A6, A7, A8, A9
<i>ada2</i>	50.23 ± 3.44	A1, B1, B2, B3, B4, B5, B6
<i>ada3</i>	50.94 ± 0.26	A2, B1, C1, C2, C3, C4, C5
<i>ada3::ADA3</i>	51.63 ± 1.54	A3
<i>gcn5</i>	53.16 ± 4.52	A4, B2, C1, D1, D2, D3, D4
<i>gcn5::GCN5</i>	49.19 ± 1.94	A5
<i>ada2 ada3</i>	55.42 ± 3.53	A6, B3, C2, D1, E1, E2, E3
<i>ada2 gcn5</i>	53.50 ± 5.72	A7, B4, C3, D2, E1, F1, F2
<i>ada3 gcn5</i>	52.66 ± 0.85	A8, B5, C4, D3, E2, F1, G1
<i>ada2 ada3 gcn5</i>	52.28 ± 4.00	A9, B6, C5, D4, E3, F2, G1

Doubling time are shown with mean ± standard error of the mean for three technical replicates. Statistical analyzed by unpaired t test with Welch's correction. The *P* values between two measurements with the same letters were as follows: A1, *P* = 0.2935; A2, *P* = 0.1028; A3, *P* = 0.0829; A4, *P* = 0.2479; A5, *P* = 0.2446; A6, *P* = 0.0855; A7, *P* = 0.3035; A8, *P* = 0.0745; A9, *P* = 0.2529; B1, *P* = 0.8557; B2, *P* = 0.6425; B3, *P* = 0.3697; B4, *P* = 0.6583; B5, *P* = 0.5649; B6, *P* = 0.7239; C1, *P* = 0.6737; C2, *P* = 0.333; C3, *P* = 0.6992; C4, *P* = 0.194; C5, *P* = 0.7706; D1, *P* = 0.7193; D2, *P* = 0.9654; D3, *P* = 0.9233; D4, *P* = 0.8938; E1, *P* = 0.7935; E2, *P* = 0.5255; E3, *P* = 0.5971; F1, *P* = 0.8974; F2, *P* = 0.8724; G1, *P* = 0.9351.

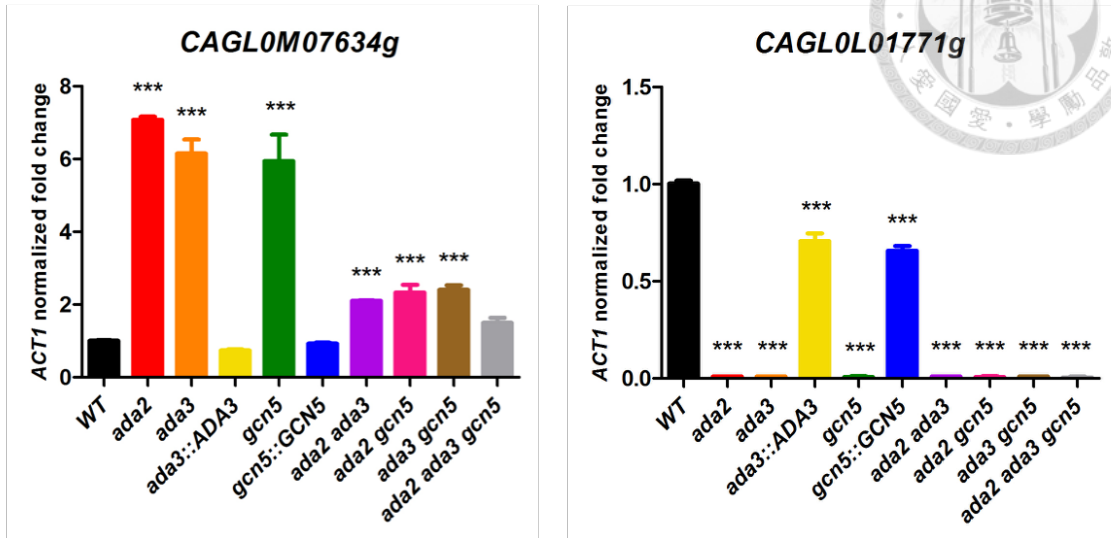


Figure S1. The expression of two pseudohyphal regulation orthologs in *C. glabrata* were affected by HAT module.

CAGL0M07634g and *CAGL0L01771g* are *SOK2* and *PHD1* ortholog in *S. cerevisiae*, respectively. Loss of *ADA2*, *ADA3* or *GCN5* in *C. glabrata* increased *CAGL0M07634g* expression level but decreased *CAGL0L01771g* expression level.

8. Future work

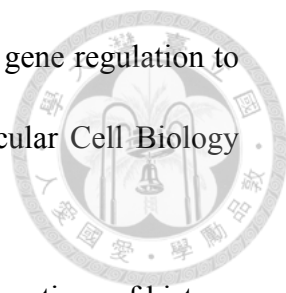
- a. Using GFP or other reporter genes to better understand the subcellular localization of Gcn5 in the absence of Ada2 and Ada3.
- b. To investigate whether defect of HAT module affects the function of other modules in SAGA complex.
- c. Using protein-protein interaction to elucidate whether other proteins function as Gcn5 will interact with SAGA complex in the absence of Gcn5.
- d. To find which transcription factor is regulated by SAGA complex against oxidative stress in *C. glabrata*.

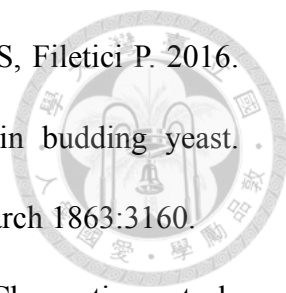


9. References


1. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. 2018. Invasive candidiasis. *Nature Reviews Disease Primers* 4:18026.
2. Tan BH, Chakrabarti A, Li RY, Patel AK, Watcharananan SP, Liu Z, Chindamporn A, Tan AL, Sun PL, Wu UI, Chen YC, Xu YC, Wang H, Sun ZY, Wang LL, Lu J, Yang Q, Zhang QQ, Shao HF, Liao K, Woo PCY, Marak RSK, Kindo AJ, Wu CL, Ho MW, Lu PL, Wang LS, Riengchan P. 2015. Incidence and species distribution of candidaemia in Asia: a laboratory-based surveillance study. *Clinical Microbiology and Infection* 21:946.
3. Wiederhold NP. 2017. Antifungal resistance: current trends and future strategies to combat. *Infection and drug resistance* 10:249.
4. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. 2012. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiology Reviews* 36:288.
5. Kumar K, Askari F, Sahu MS, Kaur R. 2019. *Candida glabrata*: A Lot More Than Meets the Eye. *Microorganisms* 7:39.
6. De Las Peñas A, Juárez-Cepeda J, López-Fuentes E, Briones-Martín-del-Campo M, Gutiérrez-Escobedo G, Castaño I. 2015. Local and regional chromatin silencing in *Candida glabrata*: consequences for adhesion and the response to stress. *FEMS Yeast Research* 15.
7. Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. *Cell Research* 21:381.



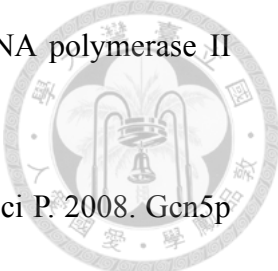
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8. Verdin E, Ott M. 2015. 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. *Nature Reviews Molecular Cell Biology* 16:258.
9. Suganuma T, Workman JL. 2011. Signals and combinatorial functions of histone modifications. *Annual Review of Biochemistry* 80:473.
10. Hong L, Schroth GP, Matthews HR, Yau P, Bradbury EM. 1993. Studies of the DNA binding properties of histone H4 amino terminus. Thermal denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 "tail" to DNA. *Journal of Biological Chemistry* 268:305.
11. Sun J, Paduch M, Kim S-A, Kramer RM, Barrios AF, Lu V, Luke J, Usatyuk S, Kossiakoff AA, Tan S. 2018. Structural basis for activation of SAGA histone acetyltransferase Gcn5 by partner subunit Ada2. *Proceedings of the National Academy of Sciences* 115:10010.
12. Balasubramanian R, Pray-Grant MG, Selleck W, Grant PA, Tan S. 2002. Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. *Journal of Biological Chemistry* 277:7989.
13. Cieniewicz AM, Moreland L, Ringel AE, Mackintosh SG, Raman A, Gilbert TM, Wolberger C, Tackett AJ, Taverna SD. 2014. The bromodomain of Gcn5 regulates site specificity of lysine acetylation on histone H3. *Molecular & Cellular Proteomics* 13:2896.
14. Wu M, Newcomb L, Heideman W. 1999. Regulation of gene expression by glucose in *Saccharomyces cerevisiae*: a role for *ADA2* and *ADA3/NGG1*. *Journal of Bacteriology* 181:4755.

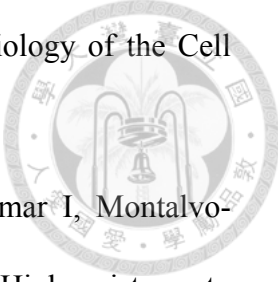
- 
15. Canzonetta C, Leo M, Guarino SR, Montanari A, Francisci S, Filetici P. 2016. SAGA complex and Gcn5 are necessary for respiration in budding yeast. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1863:3160.
 16. Kurat CF, Yeeles JTP, Patel H, Early A, Diffley JFX. 2017. Chromatin controls DNA replication origin selection, lagging-strand synthesis, and replication fork rates. *Molecular Cell* 65:117.
 17. Church M, Smith KC, Alhussain MM, Pennings S, Fleming AB. 2017. Sas3 and Ada2(Gcn5)-dependent histone H3 acetylation is required for transcription elongation at the de-repressed *FLO1* gene. *Nucleic Acids Research* 45:4413.
 18. Cormack BP, Ghori N, Falkow S. 1999. An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* 285:578.
 19. Haynes BC, Skowrya ML, Spencer SJ, Gish SR, Williams M, Held EP, Brent MR, Doering TL. 2011. Toward an integrated model of capsule regulation in *Cryptococcus neoformans*. *PLoS Pathog* 7:e1002411.
 20. Meara TR, Hay C, Price MS, Giles S, Alspaugh JA. 2010. *Cryptococcus neoformans* histone acetyltransferase Gcn5 regulates fungal adaptation to the host. *Eukaryotic Cell* 9:1193.
 21. Shivarathri R, Tscherner M, Zwolanek F, Singh NK, Chauhan N, Kuchler K. 2019. The fungal histone acetyl transferase Gcn5 controls virulence of the human pathogen *Candida albicans* through multiple pathways. *Scientific Reports* 9:9445.
 22. Chang P, Fan X, Chen J. 2015. Function and subcellular localization of Gcn5, a histone acetyltransferase in *Candida albicans*. *Fungal Genetics and Biology*


81:132.

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23. Lin C-J, Hou Y-H, Chen Y-L. 2019. The histone acetyltransferase GcnE regulates conidiation and biofilm formation in *Aspergillus fumigatus*. *Medical Mycology* doi:10.1093/mmy/myz043.
 24. Kounatidis I, Ames L, Mistry R, Ho H-l, Haynes K, Ligoxygakis P. 2018. A host-pathogen interaction screen identifies *ada2* as a mediator of *Candida glabrata* defenses against reactive oxygen species. *G3: Genes|Genomes|Genetics* 8:1637.
 25. Yu S-J, Chang Y-L, Chen Y-L. 2018. Deletion of *ADA2* Increases antifungal drug susceptibility and virulence in *Candida glabrata*. *Antimicrobial Agents and Chemotherapy* 62:e01924.
 26. Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, De Montigny J, Marck C, Neuvéglise C, Talla E. 2004. Genome evolution in yeasts. *Nature* 430:35.
 27. Reuß O, Vik Å, Kolter R, Morschhäuser J. 2004. The SAT1 flipper, an optimized tool for gene disruption in *Candida albicans*. *Gene* 341:119.
 28. Gietz D, St Jean A, Woods RA, Schiestl RH. 1992. Improved method for high efficiency transformation of intact yeast cells. *Nucleic acids research* 20:1425.
 29. Yu S-J, Chang Y-L, Chen Y-L. 2018. Deletion of *ADA2* increases antifungal drug susceptibility and virulence in *Candida glabrata*. *Antimicrobial agents and chemotherapy*:AAC. 01924.
 30. Wayne P. 2008. Reference methods for broth dilution antifungal susceptibility testing of yeasts: approved standard, 2nd ed. Document M27-A3.
 31. Workman JL, Kingston RE. 1998. Alternation of nucleosome structure as a

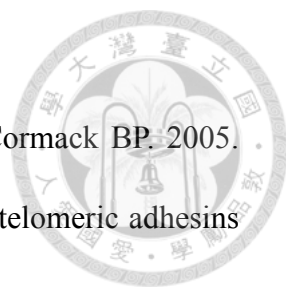
- mechanism of transcriptional regulation. *Annual Review of Biochemistry* 67:545.
32. Piña B, Berger S, Marcus GA, Silverman N, Agapite J, Guarente L. 1993. *ADA3*: a gene, identified by resistance to GAL4-VP16, with properties similar to and different from those of *ADA2*. *Molecular and Cellular Biology* 13:5981.
33. Briones-Martin-Del-Campo M, Orta-Zavalza E, Juarez-Cepeda J, Gutierrez-Escobedo G, Cañas-Villamar I, Castaño I, De Las Peñas A. 2014. The oxidative stress response of the opportunistic fungal pathogen *Candida glabrata*. *Revista Iberoamericana de Micología* 31:67.
34. Lo W-S, Dranginis AM. 1998. The cell surface flocculin Flo11 is required for pseudohyphae formation and Invasion by *Saccharomyces cerevisiae*. *Molecular Biology of the Cell* 9:161.
35. Guo B, Styles CA, Feng Q, Fink GR. 2000. A *Saccharomyces* gene family involved in invasive growth, cell–cell adhesion, and mating. *Proceedings of the National Academy of Sciences* 97:12158.
36. Rasheed M, Battu A, Kaur R. 2018. Aspartyl proteases in *Candida glabrata* are required for suppression of the host innate immune response. *Journal of Biological Chemistry* 293:6410.
37. Kaur R, Ma B, Cormack BP. 2007. A family of glycosylphosphatidylinositol-linked aspartyl proteases is required for virulence of *Candida glabrata*. *Proceedings of the National Academy of Sciences* 104:7628.
38. Helmlinger D, Tora L. 2017. Sharing the SAGA. *Trends in Biochemical Sciences* 42:850.
39. Baptista T, Grünberg S, Minoungou N, Koster MJE, Timmers HTM, Hahn S,

- 
- Devys D, Tora L. 2018. SAGA is a general cofactor for RNA polymerase II transcription. *Molecular Cell* 70:1163.
40. Vernarecci S, Ornaghi P, Bâgu A, Cundari E, Ballario P, Filetici P. 2008. Gcn5p plays an important role in centromere kinetochore function in budding yeast. *Molecular and Cellular Biology* 28:988.
41. Shih P-Y, Liao Y-T, Tseng Y-K, Deng F-S, Lin C-H. 2019. A potential antifungal effect of chitosan against *Candida albicans* is mediated via the inhibition of SAGA complex component expression and the subsequent alteration of cell surface integrity. *Frontiers in Microbiology* 10.
42. Georgakopoulos T, Gounalaki N, Thireos G. 1995. Genetic evidence for the interaction of the yeast transcriptional co-activator proteins *GCN5* and *ADA2*. *Molecular and General Genetics MGG* 246:723.
43. Brown AJP, Haynes K, Quinn J. 2009. Nitrosative and oxidative stress responses in fungal pathogenicity. *Current Opinion in Microbiology* 12:384.
44. Kasper L, Seider K, Hube B. 2015. Intracellular survival of *Candida glabrata* in macrophages: immune evasion and persistence. *FEMS Yeast Research* 15.
45. Segal AW. 2004. How neutrophils kill microbes. *Annual Review of Immunology* 23:197.
46. Galocha M, Pais P, Cavalheiro M, Pereira D, Viana R, Teixeira MC. 2019. Divergent approaches to virulence in *C. albicans* and *C. glabrata*: two sides of the same coin. *International journal of molecular sciences* 20:2345.
47. Sellam A, Askew C, Epp E, Lavoie H, Whiteway M, Nantel A. 2009. Genome-wide Mapping of the Coactivator Ada2p Yields Insight into the Functional Roles

- 
- of SAGA/ADA Complex in *Candida albicans*. *Molecular Biology of the Cell* 20:2389.
48. Cuéllar-Cruz M, Briones-Martin-del-Campo M, Cañas-Villamar I, Montalvo-Arredondo J, Riego-Ruiz L, Castaño I, De Las Peñas A. 2008. High resistance to oxidative stress in the fungal pathogen *Candida glabrata* is mediated by a single catalase, Cta1p, and is controlled by the transcription factors Yap1p, Skn7p, Msn2p, and Msn4p. *Eukaryotic Cell* 7:814.
49. Lee J, Godon C, Lagniel G, Spector D, Garin J, Labarre J, Toledano MB. 1999. Yap1 and Skn7 Control two specialized oxidative stress response regulons in yeast. *Journal of Biological Chemistry* 274:16040.
50. Morgan BA, Banks GR, Toone WM, Raitt D, Kuge S, Johnston LH. 1997. The Skn7 response regulator controls gene expression in the oxidative stress response of the budding yeast *Saccharomyces cerevisiae*. *The EMBO Journal* 16:1035.
51. Kontoyiannis DP. 1999. Genetic analysis of azole resistance by transposon mutagenesis in *Saccharomyces cerevisiae*. *Antimicrobial Agents and Chemotherapy* 43:2731.
52. Ramírez-Zavala B, Mogavero S, Schöller E, Sasse C, Rogers PD, Morschhäuser J. 2014. SAGA/ADA Complex Subunit Ada2 Is Required for Cap1- but Not Mrr1-Mediated Upregulation of the *Candida albicans* Multidrug Efflux Pump *MDR1*. *Antimicrobial Agents and Chemotherapy* 58:5102.
53. Usher J, Haynes K. 2019. Attenuating the emergence of anti-fungal drug resistance by harnessing synthetic lethal interactions in a model organism. *PLoS genetics* 15:e1008259.

- 
54. Zupan J, Raspor P. 2008. Quantitative agar-invasion assay. *Journal of Microbiological Methods* 73:100.
55. Santos R, Costa C, Mil-Homens D, Romão D, de Carvalho CCCR, Pais P, Mira NP, Fialho AM, Teixeira MC. 2017. The multidrug resistance transporters CgTpo1_1 and CgTpo1_2 play a role in virulence and biofilm formation in the human pathogen *Candida glabrata*. *Cellular Microbiology* 19:e12686.
56. Dodgson J, Avula H, Hoe K-L, Kim D-U, Park H-O, Hayles J, Armstrong J. 2009. Functional genomics of adhesion, invasion, and mycelial formation in *Schizosaccharomyces pombe*. *Eukaryotic Cell* 8:1298.
57. Hua SST, Beck JJ, Sarreal SBL, Gee W. 2014. The major volatile compound 2-phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycotoxin Research* 30:71.
58. Pukkila-Worley R, Peleg AY, Tampakakis E, Mylonakis E. 2009. *Candida albicans* hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryotic Cell* 8:1750.
59. Li D-D, Fuchs BB, Wang Y, Huang X-W, Hu D-D, Sun Y, Chai D, Jiang Y-Y, Mylonakis E. 2017. Histone acetyltransferase encoded by *NGG1* is required for morphological conversion and virulence of *Candida albicans*. *Future Microbiology* 12:1497.
60. De Las Peñas A, Pan S-J, Castaño I, Alder J, Cregg R, Cormack BP. 2003. Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to *RAP1*- and *SIR*-dependent

transcriptional silencing. *Genes & Development* 17:2245.

- 
61. Castaño I, Pan S-J, Zupancic M, Hennequin C, Dujon B, Cormack BP. 2005. Telomere length control and transcriptional regulation of subtelomeric adhesins in *Candida glabrata*. *Molecular Microbiology* 55:1246.
62. Jacobson S, Pillus L. 2009. The SAGA subunit Ada2 functions in transcriptional silencing. *Molecular and Cellular Biology* 29:6033.
63. Kristjuhan A, Wittschieben BØ, Walker J, Roberts D, Cairns BR, Svejstrup JQ. 2003. Spreading of Sir3 protein in cells with severe histone H3 hypoacetylation. *Proceedings of the National Academy of Sciences* 100:7551.
64. Gagnon-Arsenault I, Tremblay J, Bourbonnais Y. 2006. Fungal yapsins and cell wall: a unique family of aspartic peptidases for a distinctive cellular function. *FEMS Yeast Research* 6:966.
65. Forastiero A, Garcia-Gil V, Rivero-Menendez O, Garcia-Rubio R, Monteiro MC, Alastruey-Izquierdo A, Jordan R, Agorio I, Mellado E. 2015. Rapid development of *Candida krusei* echinocandin resistance during caspofungin therapy. *Antimicrobial Agents and Chemotherapy* 59:6975.