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Vacuole and mitochondria patch protein Mcp1 of *Saccharomyces boulardii* impairs the oxidative stress response of *Candida albicans* by regulating 2-phenylethanol

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Abstract

Background Vacuole and mitochondria patch (vCLAMP) protein Mcp1 is crucial in eukaryotic cells response to environmental stress, but the mechanism of Mcp1 in *Saccharomyces boulardii* (*S. boulardii*) against pathogenic fungi is unclear.

Results This work first explored the role of Mcp1 in *S. boulardii* against *Candida albicans* (*C. albicans*). The results showed that Mcp1 located on the vacuolar and mitochondrial membrane of *S. boulardii*. Overexpression of Mcp1 inhibited the adhesion and hyphal formation of *C. albicans* in vitro. The mice model of intestinal infection revealed that WT-*pGK1-MCP1* mutant enhanced the ability of *S. boulardii* antagonize *C. albicans* infecting gut. High performance liquid chromatography-mass spectrometry analysis demonstrated that overexpressing Mcp1 promoted the production of 2-phenylethanol. The latter is a secondary metabolite of *S. boulardii*, and can inhibit the adhesion and biofilm formation of *C. albicans*. The reverse transcription polymerase chain reaction and western blotting results confirmed Mcp1 promoted the production of 2-phenylethanol by regulating the expression level of Aro10. Notably, RNA-sequencing and Gene Ontology enrichment analyses showed that 2-phenylethanol impaired the oxidative stress response of *C. albicans*.

Conclusion This work reveals the critical role of Mcp1 in *S. boulardii* against *C. albicans* by regulating 2-phenylethanol metabolism, which provide a theoretical basis for *S. boulardii* as antifungal biologic therapy to prevent and treat of *Candida* infection.

Keywords Organelle membranes, Mcp1, Antagonism, 2-phenylethanol, Oxidative stress response

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Background

Candida albicans (*C. albicans*), a conditionally pathogenic fungus in clinic [1], can cause host systemic infections and even mortality in immunocompromised individuals by invading submucosal tissues of the intestine [2]. The adhesion and hyphal development of *C. albicans* play a significant role in host invasion [3]. Hitherto, drug therapy has been the classic prevention and treatment strategy for *Candida* infections. However, the limited antifungal drugs and the emergence of drug-resistant strains bring the great challenge to the clinical treatment of *Candida* infection [4, 5]. Many studies and our previous work found that the probiotic fungi-*Saccharomyces boulardii* (*S. boulardii*) can effectively inhibit virulence of *C. albicans* [6–8], and is considered as a safe and effective alternative therapy for *Candida* infections.

Recently, membrane contact site, commonly found in mammalian and yeast cells, has caused wide concern owing to their important roles in cell growth and response to environmental stresses [9, 10]. Our previous work found that vacuole and mitochondria patch (vCLAMP) proteins possess the ability to maintain mitochondrial functional stability [11–13]. Notably, overexpression of the vCLAMP protein Mcp1 in *S. boulardii* significantly enhanced the ability of probiotic fungi against *C. albicans* [6], however, the detailed mechanism has not been elucidated.

Herein, the location of vCLAMP protein Mcp1 was observed by fluorescent labeling and staining techniques. To study the functions of Mcp1 in *S. boulardii* against *C. albicans*, firstly, the adhesion and hyphal formation of *C. albicans* on polystyrene microplate were detected. Secondly, a mice gut infection model was conducted to validate the function of Mcp1 in *S. boulardii* against *C. albicans* infecting host intestines. Subsequently, the secondary metabolites of *S. boulardii* were analyzed using high performance liquid chromatography-mass spectrometry (HPLC-MS). Moreover, the reverse transcription polymerase chain reaction (RT-PCR) and western blotting were used to analyze 2-phenylethanol related gene and protein expression levels. Furthermore, RNA-sequencing (RNA-Seq) and Gene Ontology (GO) enrichment analyses were carried out to study the mechanism of 2-phenylethanol inhibiting the adhesion and biofilm formation of *C. albicans*.

Method

Construction of strains

S. boulardii, *C. albicans*, and plasmids mentioned in this work were listed in Table S1. *S. boulardii* (*ura3::ura3*) was derived from Shandong Agricultural University (Shandong Province, China). The mutants were constructed using *S. boulardii* (*ura3::ura3*) as the parental strain. WT-*pGK1-MCP1* mutant was obtained by transferring *pSP-G1-MCP1-URA3* plasmid into *S. boulardii* [14]. Amplifying *MCP1-URA3* gene knockout cassette using the primers of MCP1-5DR and MCP1-3DR, and then transformed it into *S. boulardii* to construct *mcp1*Δ/Δ mutant strain (Figure S1). The relevant primer sequences were listed in Table S2.

Microscopic analyses

WT-*pGK1-MCP1-GFP* cells were incubated in YPD medium for 12 h. Adjusting the density of cells ($OD_{600\ nm}$ = 0.1) with SC-Uri liquid medium, and then incubating cells at 30 °C for 4 h. The collected cells were stained by MitoTracker Red (dissolved in dimethyl sulfoxide, 1 mM) and observed by a fluorescence microscope. In addition, the collected cells were stained by N-(3-triethylammoniumpropyl)-4-(6-(4- (diethylamino)phenyl)hexatrienyl) pyridinium dibromide (FM4-64) (50 μg/mL, Sigma, St. Louis, MO, USA) to confirm the location of mcp1-GFP [15].

RNA extraction

C. albicans were cultured at 30 °C for 12 h with 160 rpm, and then transferred into YPD medium and cultured continuously for 4 h. Adjusting to an OD_{600} of 0.2 in RPMI-1640 medium (RP MI-1640 powder 1.04%, MOPS 0.418%, 80 μg/mL uridine, pH 7.0). After adding 2-phenylethanol with final concentration of 0.02 mg/mL, *C. albicans* were cultured for 4 h at 37 °C. RNA was extracted via Trizol method according to the previously described method [16], and reverse transcribed into single-stranded complementary DNA (cDNA) by using M-MLV reverse transcriptase. This diluted cDNA was used to the following studies.

Filamentous growth assays

C. albicans were cultured for 10 h and transferred it into YPD medium (80 μg/mL uridine) for 4 h to log phase [17]. The cells were collected by centrifugation (12000 rpm) and washed three times by PBS buffer, and suspended in RPMI-1640 medium (80 μg/mL uridine). Then, *C. albicans* were incubated with 2-phenylethanol (0.002, 0.02 and 0.2 mg/mL, respectively) at 37 °C for 4 h, and centrifuged at 12,000 rpm for 10 min to collect cells. The obtained cells were stained with fluorescent dye (calcofluor white, CFW) at a concentration of 10 mg/mL for 5 min at 30 °C, and observed using a fluorescence microscope (Leica DM2500).

Biofilm formation of *C. albicans*

The activated *C. albicans* were cultured in YPD medium (80 μg/mL uridine) for 4 h at 30 °C. Adjusting to an OD_{600} of 0.1 in RPMI-1640 medium, and transferring the cells into polystyrene microplate. The samples were cultured at 37 °C for 24 h, and stained by crystal violet to assay

the biofilm formation of *C. albicans* by multi-functional enzyme-linked immunosorbent assay reader (TECAN).

ROS assay

2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) was used to detect the intracellular ROS level in *C. albicans* [18]. After cultured at 30 °C for 6 h with 160 rpm, *C. albicans* were transferred into YPD medium containing 2-phenylethanol with final concentration of 0.02 mg/mL at 30 °C for 4 h. The cells were washed by PBS buffer and collected at 12,000 rpm for 2 min, and then stained with DCFH-DA dye (20 μg/mL) for 30 min at 30 °C. Finally, *C. albicans* were assayed by multi-functional enzyme-linked immunosorbent assay reader (Ex = 488 nm, Em = 520 nm).

Determination of intracellular ATP and cell activity

The cells cultured in YPD medium were collected for protein extraction. The intracellular ATP content in cells was detected using ATP kits. The cells cultured for 4 h was collected and adjusted to $OD_{600\ nm}$ = 0.5. MTT reagent was added to catalyze the formation of crystalline formazan from succinate dehydrogenase inside mitochondria. The latter was dissolved by dimethyl sulfoxide, and detected the absorbance of 570 nm ($OD_{570\ nm}$) by a multifunctional enzyme-linked immunosorbent assay reader.

Immunoblotting

The cells cultured in YPD medium for 12 h were collected to extract protein. A sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting were performed to analyze the cells extracts. The luminescence intensity of substrate was boosted using the specific antibody, anti-Rabbit IgG (PROMEGA W4018), anti-Tubulin (MBL PM054), and anti-GFP (MBL598). The protein imprint was imaged by a western blotting exposure device (Tanon, 4800) [17].

HPLC-MS

The supernatant from cultures of yeasts cells was collected by centrifugation, and filtered with a Millipore 0.22 μm filter. Then, the samples were analyzed by HPLC-MS (Thermo Scientific) using a C_{18} column.

Adhesion assay of *C. albicans*

To investigate the antagonistic effect of *S. boulardii* on *C. albicans*, polystyrene microplate was used to observe the adhesion of *C. albicans*. Using RPMI-1640 medium (pH 7.0) as culture medium, a 1:1 mixture of *S. boulardii* and *C. albicans* was inoculated into polystyrene well plate, and cultured at 37 °C. The unattached *C. albicans* was washed away by PBS buffer. The adhesion of *C. albicans* was determined using crystal violet staining method.

Virulence assay

The mice were placed at a constant temperature of 24 °C, and subjected to a 12 h light/dark cycle. They were fed and drank freely at the Experimental Animal Center of Shandong First Medical University. We tried our best to reduce the suffering and the number of animals in experiments.

Four-week-old C57 BL/6 female SPF mice (Jinan Pengyue Experimental Animal Breeding Co., Ltd) were randomly divided into several groups with 10 rats each. The *C. albicans* (BWP17[a]) infection was developed according to previous report with some modifications [19]. Briefly, Penicillin (1.5 mg/mL) and Streptomycin (2 mg/mL) in drinking water was given to mice beginning 3 days before infection, and continued in the entire experiment. Gastric infusion was performed to inoculate *C. albicans* (a cell suspension of 1×10^8 cells/mL). After the second day of *C. albicans* infection in mice, 200 uL of *S. boulardii* (WT, *mcp1*Δ/Δ, and WT-*pGK1-MCP1*) were continuously conveyed to the stomach for 7 d, with a cell concentration of 1×10^8 cells/mL. Eight days after *Candida* infection, the colon in mice were taken and fixed overnight in 4% formalin for observation of sections stained by hematoxylin and eosin.

Results

Deletion of Mcp1 damaged the mitochondrial morphology and functions of *S. boulardii*

In our previous reported, the vacuole and mitochondria patch (vCLAMP) proteins locate at the membrane junction of the vacuole and mitochondrion. Herein, green fluorescent labelled-Mcp1 (Mcp1-GFP) was constructed to observe the intracellular location of Mcp1. The fluorescent results suggested that Mcp1-GFP was localized on the FM4-64 labelled vacuolar membrane, as well as localized on the MitoTracker Red labelled mitochondrial membrane (Fig. 1A). In normal cells, mitochondria were in a filamentous shape. Deletion of Mcp1 caused the mitochondrial morphology changing from linear to fragmented (Fig. 1B). Moreover, ATP and MTT results further showed that Mcp1 deficiency led to mitochondrial dysfunction in *S. boulardii* (Fig. 1C and D). In addition, to evaluate the damage degree of mitochondrion, the expression level of mitochondrial DNA (mtDNA) encoding genes (*COX2*, *CIM1*, and *ATP6*), an important indicator for mitochondrial function, were detected [20]. As shown in Figure S2, deletion of *MCP1* gene decreased the mtDNA quantity of *S. boulardii*.

Mcp1 is essential in *S. boulardii* against C. *albicans*

Recent studies found that probiotic fungi can inhibit the invasion of *C. albicans*. Herein, overexpression of Mcp1 of *S. boulardii* effectively reduced the adhesion and biofilm formation ability of *C. albicans* in vitro (Fig. 2A and

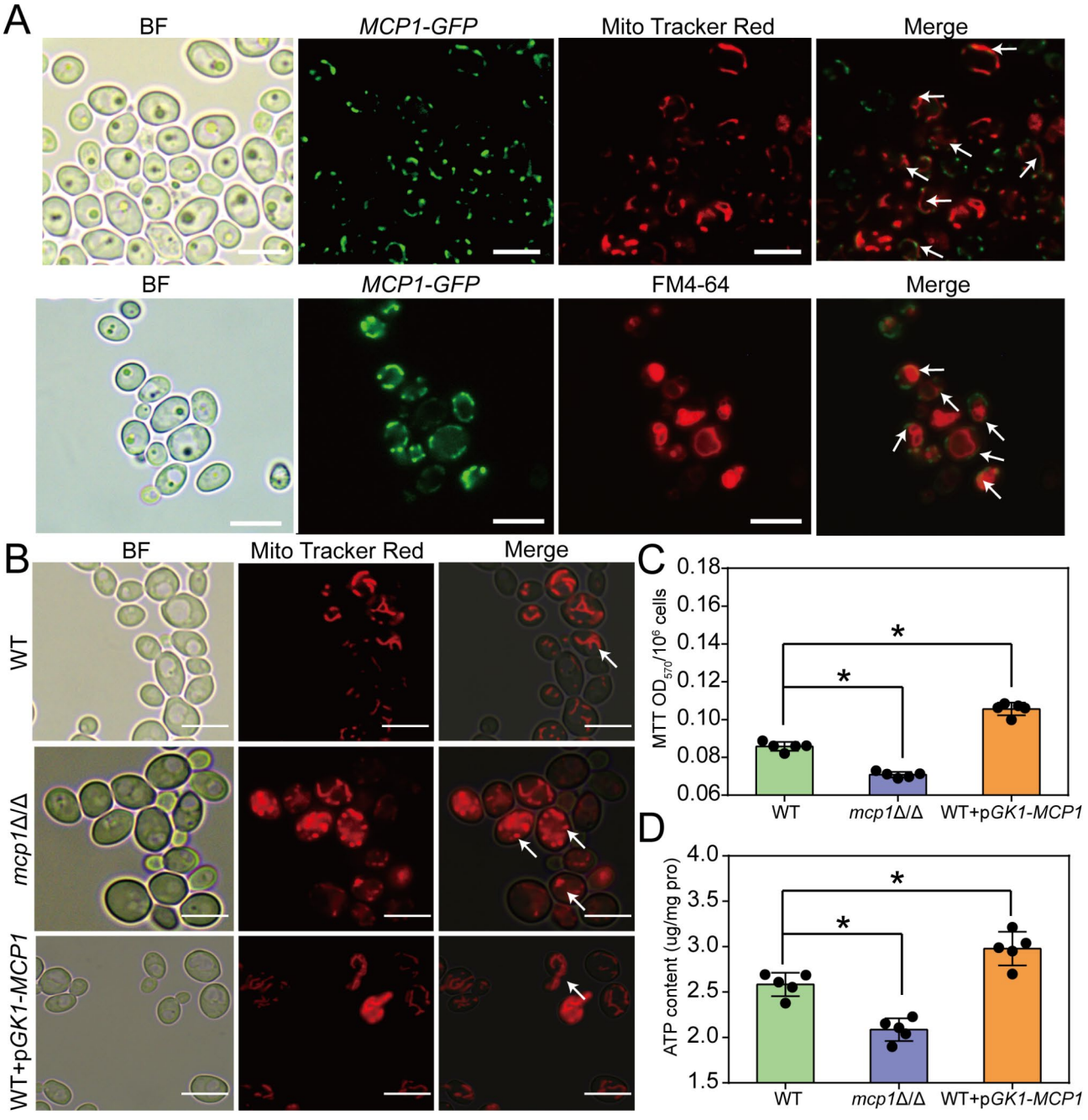

Fig. 1 Delection of *MCP1* damaged mitochondrial functions of *S. boulardii*. (**A**) Mcp1-GFP both located on vacuolar and mitochondrial membrane. After cultured in SC-Uri, the cells were collected and stained by Mito Tracker Red and FM4-64 and observed by fluorescence microscope. Bar = 5 μm. (**B**) The morphology of mitochondria in *S. boulardii* (WT, *mcp1*Δ/Δ, and WT + *pGK1-MCP1*). The samples were stianed by Mito Tracker Red. Bar = 5 μm. Deletion of *MCP1* decreased (**C**) cellar activity and (**D**) ATP production of *S. boulardii*. After the stains were mixed with MTT at 30℃ for 1 h, then the samples were resuspended in DMSO, and measured at OD_{570nm} by Multi functional enzyme-linked immunosorbent assay reader. The intracellular ATP contents were detected using an ATP kit. This value represents the mean ± SD from three replicates. Statistical significance was defined based on the different *p*-values: **p* < *0.05*, ***p* < *0.01*, and ****p* < *0.001*, ns: The comparison between the two sets of data was not statistically significant

B). To demonstrate the biosafety of *S. boulardii* used in vivo, an oral-gavage method was applied to deliver *S. boulardii* to mice. As shown in Fig. 2C, the heart, liver, lung, and kidney tissues of mice after oral-gavage treatment were similar to that of normal groups, indicating that *S. boulardii* is harmless to the body.

Subsequently, a mice gut infection model was conducted to validate the function of Mcp1 in *S. boulardii* against C. *albicans*. The mice colon was stained using

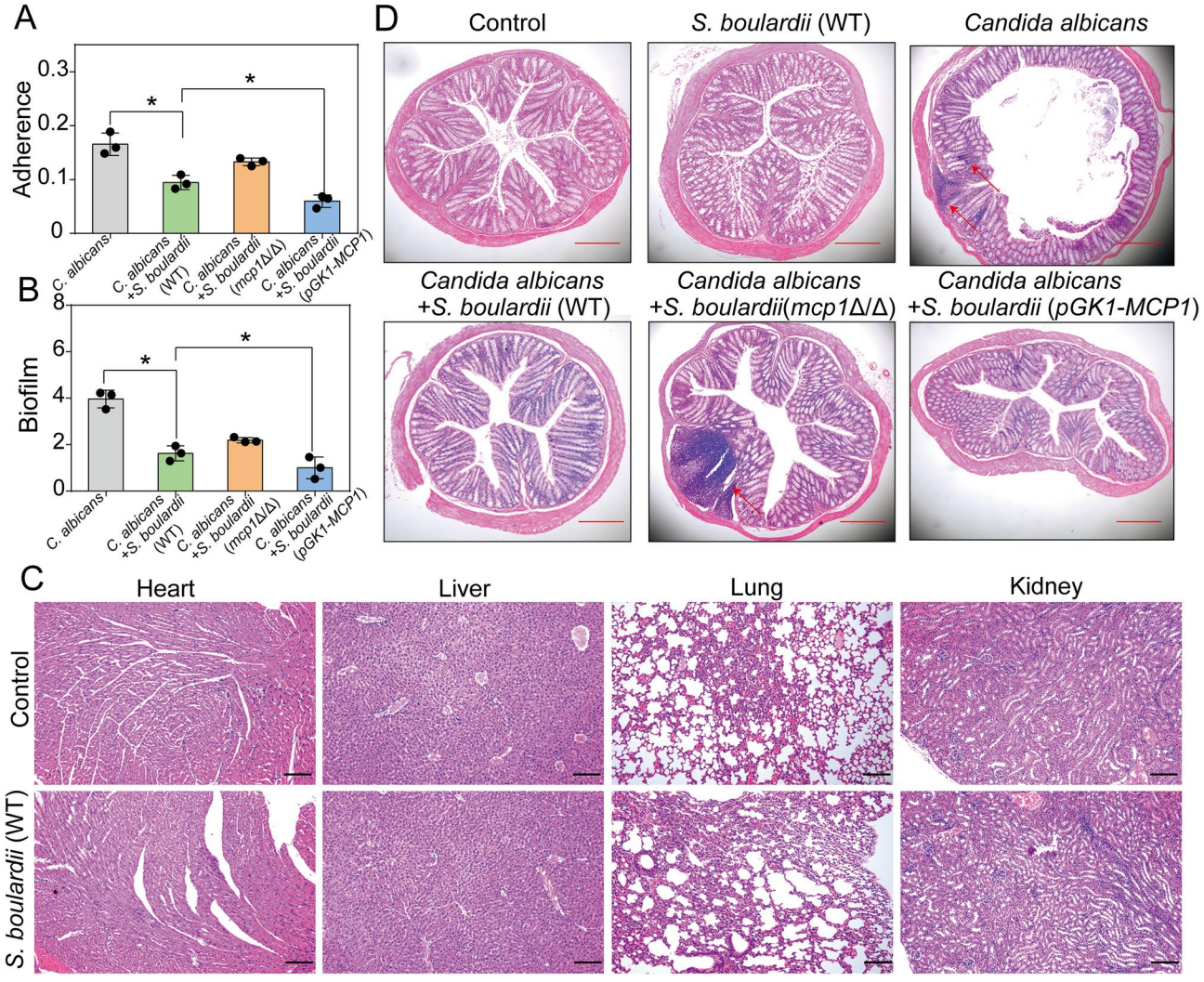

Fig. 2 Overexpression of Mcp1 of *S. boulardii* inhibited the (**A**) adhesion and (**B**) biofilm formation of *C. albicans*. (**C**) The heart, liver, lung, and kidney tissues of mice by H&E staining after oral-gavage treatment. Bar = 50 μm. (**D**) Overexpression of Mcp1 reduced the ability of *C. albicans* infecting the host gut. Bar = 50 μm. This value represents the mean ± SD from three replicates. Statistical significance was defined based on the different *p*-values: **p* < *0.05*, ***p* < *0.01*, and ****p* < *0.001*, ns: The comparison between the two sets of data was not statistically significant

hematoxylin and eosin staining (H&E) staining method. HE staining results showed that overexpression of Mcp1 in *S. boulardii* significantly reduced the intestinal invasion ability of *C. albicans* (Fig. 2D). These results demonstrated that Mcp1 of *S. boulardii* plays an important role in the antagonism process of *C. albicans* infecting host.

Mcp1 plays an important role in *S. boulardii* against *C. albicans* by regulating 2-phenylethanol

As reported, the secondary metabolites of probiotics play a major role in the antagonism of pathogenic microorganisms. In present work, we found that the secondary metabolites of *S. boulardii* inhibited the adhesion and biofilms formation of *C. albicans* (Fig. 3A, B, and C). After co-cultured with *C. albicans*, the secondary metabolites of *S. boulardii* were analyzed using HPLC-MS (Figs. 3D and S3). The results suggested that overexpression of Mcp1 significantly increased the production of 2-phenylethanol, and deletion of Mcp1 declined the production of 2-phenylethanol of *S. boulardii* (Fig. 3E).

Further studies found that 2-phenylethanol effectively inhibited the adhesion and hyphal formation of *C. albicans* (Fig. 3F and G). Notably, the hyphal length of *C. albicans* in the treated and untreated groups were analyzed by Image J. As shown in Figure S4, the hyphal length of treated samples gradually decreased as the concentration of 2-phenylethanol increased from 0.002 to 0.2 mg/mL. The hyphal length of control group was more than five times that of the treated samples which co-cultured with 2-phenylethanol at 0.2 mg/mL. Based on these results, we speculate that Mcp1 of *S. boulardii* may antagonize *C. albicans* by regulating 2-phenylethanol

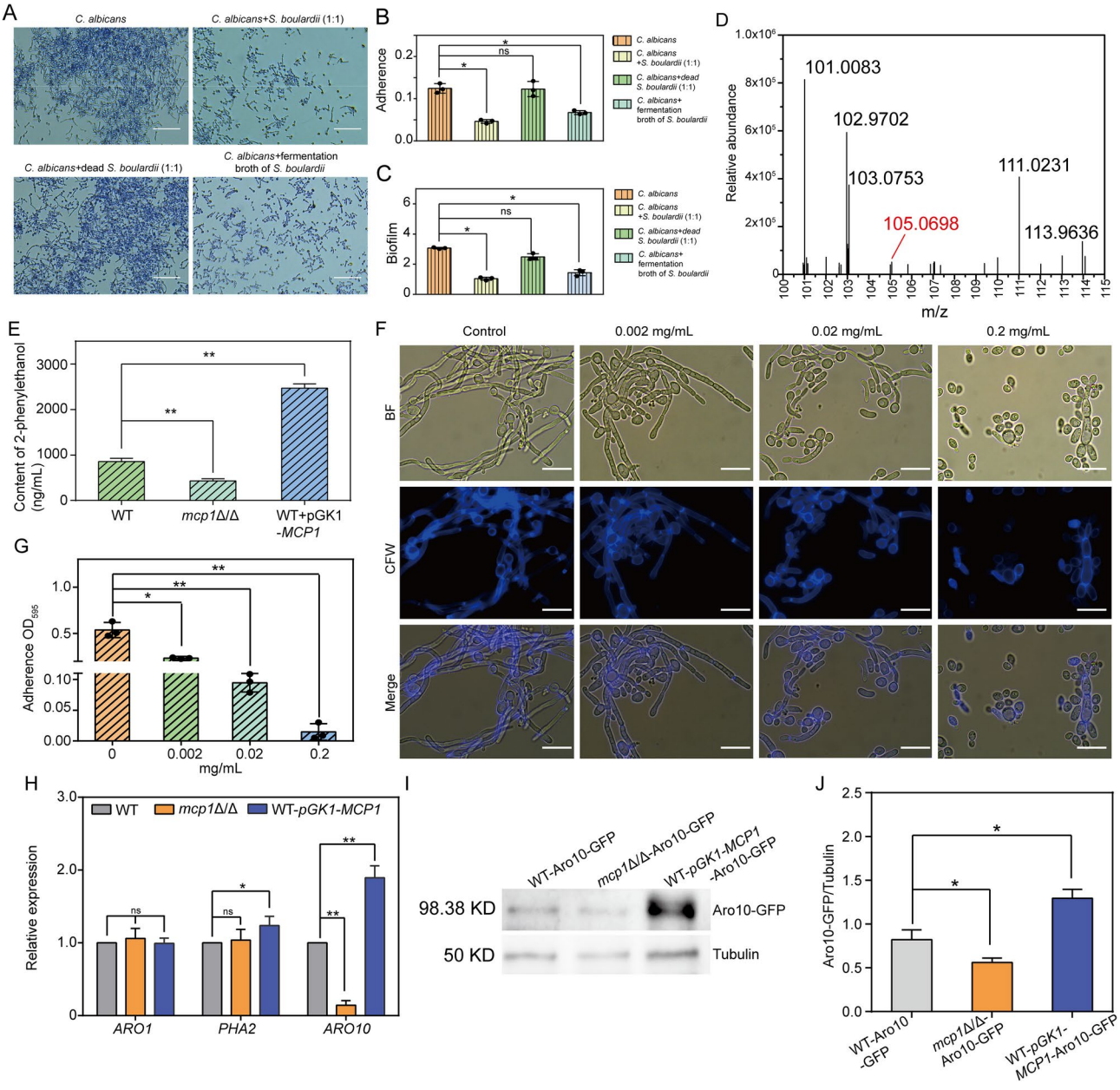

Fig. 3 The secondary metabolites of *S. boulardii* inhibited the (**A** and **B**) adhesion and (**C**) biofilm formation of *C. albicans*. (**D**) The characteristic peak of 2-phenylethanol is 105.0698 (*m/z*). (**E**) Characteristic of 2-phenylethanol content in secondary metabolites of *S. boulardii*. Cells (WT, *mcp1*Δ/Δ, and WT-*pGK1-MCP1*) cultured medium were collected, measured and analyzed by HPLC-MS. (**F**) 2-phenylethanol inhibited hyphal development of *C. albicans*. After *C. albicans* cultured in YPD at 30℃, the cells were collected and resuspended in RPMI-1640 medium with 2-phenylethanol (0.002 mg/mL, 0.02 mg/mL, and 0.2 mg/mL) at 37℃ for 4 h. Then, the samples were stained by CFW, and observed by microscope. Bar = 10 μm. (**G**) 2-phenylethanol reduced the adhesion ability of *C. albicans*. (**H**) Deletion of *MCP1* decreased the gene expression level of *ARO10*. The genes related to the metabolism of 2-phenylethanol (*ARO1*, *PHA2*, and *ARO10*) were analyzed by RT-PCR using *ACT1* as the normalization genes. (**I**) Deletion of *MCP1* led to abnormal expression of Aro10 in *S. boulardii*. The cells were collected, and the total protein was extracted. After the samples were detected by western blotting using anti-GFP and anti-tubulin antibodiers. The results were presented through an exposure device. (**J**) Gray value analysis of Aro10 by Image J. This value represents the mean ± SD from three replicates. Statistical significance was defined based on the different *p*-values: **p* < 0.05, ***p* < 0.01, and ****p* < 0.001, ns: The comparison between the two sets of data was not statistically significant

metabolism. To verify our hypothesis, RT-PCR and western blotting were used to detect the expression levels of key proteins involved in 2-phenylethanol metabolism. RT-PCR results suggested that overexpression of Mcp1 increased the expression level of *ARO10* (Fig. 3H). Many literatures reported that Aro10 is a key catalytic protein in 2-phenylethanol production [21, 22]. Western blotting results further showed that the protein expression level

of Aro10 was enhanced by overexpressing Mcp1 (Fig. 3I and J and S5). These results suggested that Mcp1 plays an important role in *S. boulardii* against *C. albicans* by regulating 2-phenylethanol.

Mcp1 of *S. boulardii* damage the oxidative stress response of *C. albicans* by regulating 2-phenylethanol

To investigate the mechanism of Mcp1 inhibited the adhesion and biofilm formation of *C. albicans* by regulating 2-phenylethanol, the intracellular RNA expression levels of *C. albicans* after 2-phenylethanol treatment were analyzed using RNA-sequencing (RNA-Seq). Transcriptomic analysis showed that 3162 genes (1458 upregulated and 1704 downregulated) were differentially expressed, suggesting that 2-phenylethanol treatment caused a substantial difference in gene expression of *C. albicans* (Fig. 4A). Gene Ontology (GO) enrichment analyses further suggested that 2-phenylethanol led to abnormalities in oxidative stress response of *C. albicans* (Fig. 4B). The expression level of genes related to oxidative stress response, such as *TRR1*, *SOD1* and *CAT1* were analyzed by RT-PCR. As shown in Figs. 2 and 4C-phenylethanol treatment resulted in an obvious decrease in the expression levels of *TRR1*, *SOD1* and *CAT1*. These results suggested that 2-phenylethanol may damage the oxidative response process of *C. albicans.*

Moreover, the expression level of hypha-associated genes including *ALS1*, *ALS3* and *HWP1* in *C. albicans* were also detected by RT-PCR. As expected, the addition of 2-phenylethanol resulted in a significant decrease in the expression levels of the aforementioned hypha-associated genes (Fig. 4D). Furthermore, 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining revealed that the *C. albicans* exhibited higher intracellular reactive oxygen species (ROS) after treated by 2-phenylethanol (Fig. 4E). In addition, the determination of antioxidant enzyme activity showed that the activity of superoxide dismutases (SOD) and catalase (CAT) declined obviously (Fig. 4F and G). To verify that 2-phenylethanol generated the oxidative stress in *C. albicans*, the *cat1*Δ/Δ and p*ACT1-CAT1* strains were constructed. Compared with WT, the ROS level in *cat1*Δ/Δ increased, but overexpressing *CAT1* decreased the ROS in *C. albicans*. Moreover, the addition of 2-phenylethanol intensified the level of intracellular ROS in *cat1*Δ/Δ (Fig. 4H). These results implied that 2-phenylethanol regulated by Mcp1 of *S. boulardii* damaged the oxidative stress response of *C. albicans*.

Discussion

C. albicans, a common conditionally pathogenic fungus in clinical practice, easily cause host systemic infections and even endanger life by invading submucosal tissues [23]. The adhesion and hyphal development capabilities of *C. albicans* play important roles in the process of invading the host [24]. Nowadays, the treatment of *Candida* infection mainly relies on medication. However, the limited clinical antifungals and the emergence of drug-resistant *C. albicans* highlight the challenges in the antifungal treatments [25].

S. boulardii, a probiotic fungi isolated from fruits, can effectively inhibit the colonization of *C albicans* in host intestine [8]. As reported, the vCLAMP mediated by Mcp1 is a novel organelle membrane junction structure. It facilitates information exchange between organelles, and effectively restores the mitochondrial damage caused by ERMES deficiency. Our previous work constructed a *S. boulardii* bio-coating based on microbial confined growth technology [6]. Interestingly, we found that overexpression of vCLAMP protein Mcp1 of *S. boulardii* in bio-coating diminished the adhesion and biofilm formation abilities of *C. albicans*. Overexpression of Mcp1 enhanced antagonistic ability of *S. boulardii* against the process of *C. albicans* infecting mice intestine. These studies suggested that Mcp1 plays an important role in *S. boulardii* against *C. albicans*, but the detailed mechanism is unclear.

Latest studies found that the secondary metabolites of probiotics could inhabit the growth and reproduction of pathogenic microorganisms [26]. To clarify the mechanism of Mcp1 in *S. boulardii* against *C. albicans*, the secondary metabolites of *S. boulardii* (WT, *mcp1*Δ/Δ, and WT-*pGK1-MCP1*) were analyzed using HPLC-MS. The results found that overexpression of Mcp1 significantly enhanced the production of 2-phenylethanol in the secondary metabolites of *S. boulardii*. Most importantly, 2-phenylethanol reduced the adhesion and hyphal formation of *C. albicans*.

To validate Mcp1 plays an essential role in *S. boulardii* against *C. albicans* by regulating 2-phenylethanol, the expression levels of 2-phenylethanol metabolism related genes (*ARO1*, *PHA2* and *ARO10*) were detected. RT-PCR results found that overexpression of Mcp1 enhanced the gene expression level of Aro10. As reported, Aro10 is one of the most widely available phenylpyruvate decarboxylase involved in 2-phenylethanol production [21, 22]. Western blotting results further showed that overexpression of Mcp1 enhanced the protein expression level of Aro10 in *S. boulardii*. These results suggested that Mcp1 may promote the production of 2-phenylethanol by regulating the expression level of Aro10, and then enhanced the ability of *S. boulardii* against *C. albicans*.

To further study the mechanism of 2-phenylethanol inhibit the adhesion and biofilm formation of *C. albicans*, RNA-Seq and GO enrichment analyses were used to analyze RNA expression levels of *C. albicans* after 2-phenylethanol treatment. The results suggested that 2-phenylethanol may damage the oxidative

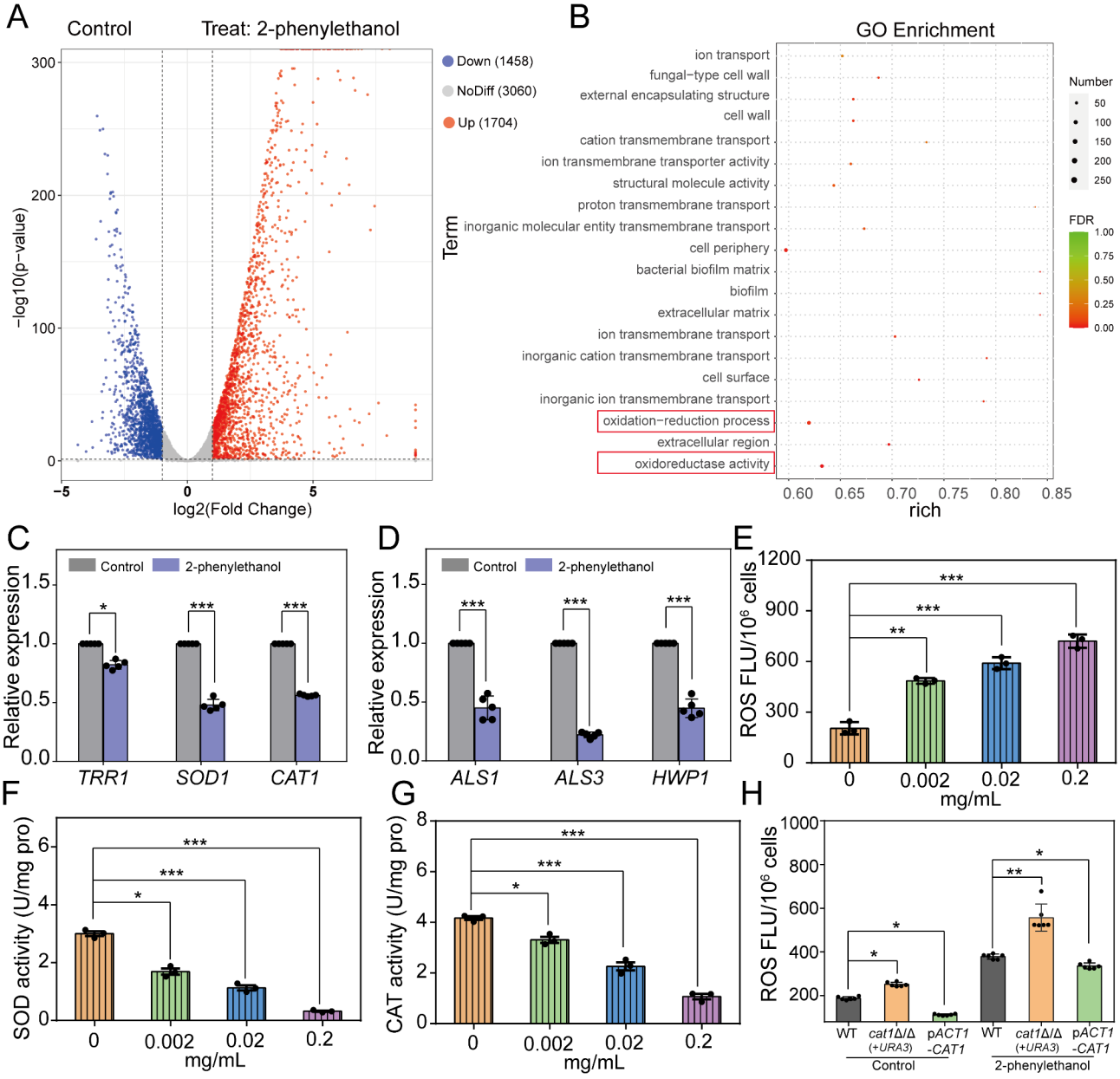

Fig. 4 (**A**) Transcriptomic analysis of differential gene expression of *C. albicans*. After the *C. albicans* was cultured with 2-phenylethanol, the genes expression of *C. albicans* were detected by RNA-seq. (**B**) GO enrichment analyses of differential gene expression. (**C**) After cultured with 2-phenylethanol, the expression level of genes related to oxidative stress response in *C. albicans*, including *TRR1*, *SOD1* and *CAT1* were assayed by RT-PCR. (**D**) The expression level of hypha-associated genes including *ALS1*, *ALS3* and *HWP1* in *C. albicans* after cultured with 2-phenylethanol. (**E**) ROS level, (**F**) SOD activity, and (**G**) CAT activity in *C. albicans* after cultured with 2-phenylethanol. (**H**) The impact on intracellular ROS levels by deleting and overexpressing *CAT1* in *C. albicans*. This value represents the mean ± SD from three replicates. Statistical significance was defined based on the different *p*-values: **p* < 0.05, ***p* < 0.01, and ****p* < 0.001, ns: The comparison between the two sets of data was not statistically significant

stress response in *C. albicans*. RT-PCR results showed that 2-phenylethanol treatment resulted in an obvious decrease in the expression levels of *TRR1*, *SOD1* and *CAT1*. As well known, antioxidant enzymes (such as SOD and CAT) in organisms can protect cells from oxidative damage by removing ROS. Determination of antioxidant enzyme activity showed that the activity of SOD and CAT significantly reduced. Furthermore, compared with WT, the ROS level in *cat1*Δ/Δ increased, but overexpressing *CAT1* decreased the ROS in *C. albicans*. Moreover, the addition of 2-phenylethanol intensified the level of intracellular ROS in *cat1*Δ/Δ. These results indicated that Mcp1 of *S. boulardii* damaged the oxidative stress response of *C. albicans* by regulating 2-phenylethanol.

Conclusions

This study revealed that vCLAMP protein Mcp1 promotes the production of 2-phenylethanol by regulating the expression level of Aro10. Interestingly, 2-phenylethanol can damage the oxidative stress response of *C. albicans*, and then inhibit the adhesion, hyphal formation, and intestinal infection of *C. albicans*. Therefore, we can infer that Mcp1 of *S. boulardii* damage the oxidative stress response of *C. albicans* by regulating 2-phenylethanol. This work enriches the function cognition of organelle membrane junction proteins, and provides new idea for the development of safe and economical antifungal strategy in clinic.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-025-02721-0.

Supplementary Material 1

Author contributions

Y.W. and X.Z. conducted the experiments. C.L., J.F. and W.G. analyzed the data. Y.W. and X.M. prepared the manuscript draft. Y.W. and X.M. designed the whole project.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shandong First Medical University & Shandong Academy of Medical Sciences (permit number W202302270104).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare no competing interests.

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