Abstract

Hsp12p is a small heat shock protein and conserved among fungal species. To investigate the expression of this heat shock protein in the fungal pathogen Candida albicans we developed an anti-CaHsp12p antibody. We show that this protein is induced during stationary phase growth and under stress conditions including heat shock, osmotic, oxidative and heavy metal stress. Furthermore, we find that CaHsp12p expression is influenced by the quorum sensing molecule farnesol, the change of CO₂ concentration and pH. Notably we show that the key transcription factor Efg1p acts as a positive regulator of CaHsp12p in response to heat shock and oxidative stress and demonstrate that CaHsp12p expression is additionally modulated by Hog1p and the cAMP-PKA signaling pathway. To study the function of Hsp12p in C. albicans we generated a null mutant, in which all four CaHSP12 genes have been deleted. Phenotypic analysis of the strain shows that CaHSP12 is not essential for stress resistance, morphogenesis or virulence when tested in a Drosophila model of infection. However, when overexpressed, CaHSP12 significantly enhanced cell-cell adhesion, germ tube formation and susceptibility to azole antifungal agents whilst desensitizing C. albicans to the quorum sensing molecule farnesol.

Introduction

Candida albicans is an ascomycete yeast which can be found in the gastrointestinal tract and the oral or vaginal mucosa of many otherwise healthy individuals [1]. However, it is also a major opportunistic fungal pathogen, causing superficial infections of mucosa and skin, or life-threatening invasive infections when either the innate or acquired immune system is compromised [1,2].

Prompt sensing and adaptation to a wide range of environmental conditions are key for fungal survival in the various niches of the host and C. albicans has developed a fine-tuned response to stresses required for efficient host colonization [3,4,5,6,7]. The ability to undergo a reversible morphological transition in response to environmental changes is an additional advantage of C. albicans host adaptation. In fact, both stress response and polymorphism are considered major virulence factors of C. albicans [8,9]. Previous studies have shown that the Hog1p MAPK signaling pathway plays an important role in regulation of stress response [8] whereas C. albicans polymorphism is controlled by multiple signaling pathways including the Efg1p-mediated cAMP pathway, Cph1p-mediated MAPK, Rim101p and Tup1p dependent pathways [6,10,11,12].

Heat shock proteins, a group of molecular chaperones found in all organisms, accumulate upon a shift from physiological to higher temperatures. They are also induced by other stresses and thus allow the cells to survive under challenging conditions [13]. Heat shock proteins are classified based on their molecular weight [14]. Small heat shock proteins have a molecular mass ranging between 10 and 30 kDa and share a conserved sequence in their C-terminus called the α-crystallin domain [15,16]. Yeast small heat shock proteins, including Hsp26p and Hsp30p, are induced under heat shock and during stationary growth phase [17,18]. Overexpression of Hsp26p increases thermo-tolerance of yeast cells [19] and C. albicans Hsp30p has been shown to be induced upon exposure to the antifungal agent amphotericin B [20].

C. albicans HSP12 (CaHSP12) gene expression is regulated by changes in the concentrations of environmental CO₂ and pH via the cAMP-dependent and Rim101p-dependent signaling cascades [21]. Other reports have shown that CaHSP12 is induced when cells are exposed to osmotic stress, oxidative stress, heavy metal stress and heat shock [8,22,23]. Additionally, CaHSP12 expression is regulated by quorum sensing molecules [24], upon hypoxic conditions [25], drug-resistance [26,27], tissue invasion [28], the yeast-to-hyphal transition [29] and iron limitation [30]. HSP12 in Candida glabrata, which is the second most common cause of systemic candidiasis, is up-regulated in fluconazole-resistant mutants [31]. Finally, HSP12 orthologs in Cryptococcus neoformans which is another pathogenic fungus have role in polyene antifungal drug susceptibility and are regulated by the cAMP signaling pathway [32].
Despite this large amount of information gathered on HSP12 gene expression very little is known about its function in fungal species in general and nothing on the C. albicans Hsp12p protein in particular. In this study, we characterize CaHSP12 from the fungal pathogen C. albicans with respect to its gene structure, regulation of protein expression, function and virulence. We show that CaHsp12p is induced by stress and the quorum sensing molecule farnesol, and regulated by the change of CO2 concentration and pH. Notably, we identify the transcription factor Efg1p to be required for expression in response to heat shock and oxidative stress in particular. We find that Hsp12p is not essential for stress resistance, filamentation or virulence. However, when overexpressed, it enhances cell-cell aggregation, susceptibility to azole antifungal agents, and promotes farnesol tolerance.

Materials and Methods

Strains and growth conditions

The yeast strains used in this study are listed in Table 1. All strains were grown in rich YEPD medium or in YNB minimal medium buffered with 150 mM HEPES as described [5,21]. All C. albicans and C. glabrata strains were grown at 37°C unless indicated otherwise. All S. cerevisiae strains were grown at 30°C.

| Strain | Description | Genotype | Source |
|--------|-------------|----------|--------|
| SC5314 | C. albicans laboratory wild-type strain | ura3::imm434/ura3::imm434 | [33] |
| CA4   | URA3 auxotrophic strain | ura3::imm434/ura3::imm434 | [33] |
| BWP17 | URA3, HIS1, ARG4 auxotrophic strain | ura3::imm434/ura3::imm434 hisG/hisG hisG/hisG arg4/arg4 hisG | [34] |
| CA4-pFM2 | Wild-type strain transformed with pFM2 as the control in HSP12 overexpression experiment | ura3::imm434/ura3::imm434 (pFM2 URA3) | This study |
| BWT   | With-type strain transformed with Clp30 as the control in CaHSP12 deletion experiment | ura3::imm434/ura3::imm434 hisG/hisG hisG/hisG arg4/arg4 hisG rps1-(Clp30 URA3, HIS1, ARG4) | This study |
| HSP12OE | CaHSP12 overexpressing strain | ura3::imm434/ura3::imm434 (CaHSP12-pFM2 CaHSP12, URA3) | This study |
| HSP12KO2 | Strain with two CaHSP12 alleles deleted | ura3::imm434/ura3::imm434 hisG/hisG hisG/hisG arg4/arg4 hisG/arg4 hisG/arg4 hisG rps1-(CaHSP12-Clp30 CaHSP12, URA3, HIS1, ARG4) | This study |
| HSP12KOS | CaHSP12 deletion strain transformed with Clp30 | ura3::imm434/ura3::imm434 hisG/hisG hisG/hisG arg4/arg4 hisG/arg4 hisG/arg4 hisG/arg4 hisG/arg4 hisG rps1-(CaHSP12-Clp30 CaHSP12, URA3, HIS1, ARG4) | This study |
| HSP12C | CaHSP12 reconstitution strain | ura3::imm434/ura3::imm434 hisG/hisG hisG/hisG arg4/arg4 hisG/arg4 hisG/arg4 hisG/arg4 hisG/arg4 hisG/arg4 hisG/arg4 hisG rps1-(CaHSP12-Clp30 CaHSP12, URA3, HIS1, ARG4) | This study |
| Cg2001 | C. glabrata wild-type | arg4/arg4 | [38] |
| Cg2001TU | C. glabrata TRP1 URA3 auxotrophic strain | arg4/arg4 trp1 | [38] |
| Cg12K0 | CgHSP12 deletion strain | arg4/arg4 trp1 CgHSP12::TRP1 (pEM13D URA3) | This study |
| Cg12C | CgHSP12 reconstitution strain | arg4/arg4 trp1 CgHSP12::TRP1 (pEM13D CgHSP12, URA3) | This study |
| BY4741 | S. cerevisiae HIS3 LEU2 MET15 URA3 auxotrophic strain | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | [63] |

Strain construction

For a comprehensive description of all methods see Text S1. Briefly, both HSP12 loci (designated CaHSP12a and CaHSP12b) present in the C. albicans genome were deleted by using Ura-blaster and HIS1 cassettes in BWP17 strain according to standard protocols [33,34,35]. Reconstitution strains (HSP12C) were constructed by integrating Clp30 containing a wild-type copy of CaHSP12 to the RP10 locus [36]. To construct the CaHSP12 overexpressing strain, HSP12OE, CaHSP12 was cloned downstream of the TEF2 promoter in pFM2 [3]. C. glabrata HSP12 was cloned, disrupted and reconstituted according to standard protocols [37,38].

Anti-CaHsp12p antibody generation

CaHsp12p was expressed in E. coli and purified using GST-tag affinity chromatography. Purified CaHsp12p was then boiled at 95°C for 10 min before sending to Charles River Laboratories (Romans-sur-Isère, France). Antibody generation, protein expression in yeast and Western blotting are detailed in Text S1 and as previously described [5].

Phenotypic assays

Growth rate determination, cell-cell aggregation and adhesion studies using the XTT reduction assays, farnesol susceptibility studies, antifungal drug and stress sensitivity tests and virulence test using our previously published Drosophila model [7] are all describe in Text S1.
Results

C. albicans contains two CaHSP12 genes

We identified two loci of CaHSP12, arranged in an inverted manner (CaHSP12a and CaHSP12b) with GenBank Accession Nos. XM715434 and XM709485, in the C. albicans genome database. Both genes are located within 6.5 kb of each other in proximity to the centromere on chromosome 5. Their predicted open reading frames encode proteins that differ in only two amino acids. The homology of the upstream regions (1640 bp) of the two different loci is 99%. However, the 1000 bp downstream regions are only <50% identical. Due to this dissimilarity, different sizes of NheI-PucI digested fragments of the two CaHSP12 copies (3.4 kb and 2.1 kb) are obtained and visualized in Southern blots (Figure 1A). We confirmed that not only the C. albicans type strain, SC5314, but equally five additional clinical isolates carry two loci of different CaHSP12 (Figure 1A). Database sequences show that there are two HSP12 genes present in the genome of Candida dubliniensis, which is closely related to C. albicans, but only one in S. cerevisiae, C. glabrata, Candida tropicalis, Candida guilliermondii, Candida lusitaniae and Cryptococcus neoformans. To determine if both copies of CaHSP12 are expressed, qRT-PCR was carried out in a strain in which one copy of CaHSP12 had been deleted (HSP12KO2). This showed a reduction of CaHSP12 expression by 50% compared to the parent strain (Figure 1B) suggesting that both copies are expressed in C. albicans.

C. albicans HSP12 contains two putative start codons

Bioinformatics analysis identified two putative start codons (ATG) for both CaHSP12 loci while only one is found in HSP12 from other fungal species. Translation from the first start codon would produce a 168 amino acid protein corresponding to a 18.0 kDa protein whereas translation from the second would lead to a 127 amino acid, 13 kDa protein. The origin of transcription was determined by analysis of the 5’ end of CaHSP12 mRNA via sequence analysis of 5’ RACE reaction products (Figure 1C). This revealed that the 5’ start point of the CaHSP12 transcript is present at position −29 from the second start codon (Figure 1C). Western blot analysis, using an anti-Hsp12p antibody, identified a signal with a size of 13 kDa (Figure 2A).

C. albicans Hsp12p is regulated in response to a wide range of stresses

To study C. albicans cells that were exposed to different stresses we raised an anti-CaHsp12p antibody (Figure 2A). Furthermore, we compared the expression of Hsp12p between C. albicans and S. cerevisiae using an S. cerevisiae anti-Hsp12 antibody. Finally, we studied the expression of CgHSP12 in C. glabrata, which is phylogenetically closely related to S. cerevisiae.

Heat shock proteins in fungi are synthesized at high levels during stationary phase growth [14,17,39] and we show that protein expression of CaHsp12p is highly induced in stationary phase but not in exponentially growing C. albicans (Figure 2B). We also show that Hsp12p is increased in stationary phase (Figure 2B), confirming previous northern blot analysis of ScHSP12 transcript by Prackelt and Meacock [14].

Transcription of HSP12 has been shown to be induced under stress in both S. cerevisiae and C. albicans [8,14,22,23,40,41]. Hence, we examined the response of Hsp12p to stress at the protein level. Western blot analysis showed that CaHsp12p is induced by heat shock (from 30°C to 45°C or from 37°C to 45°C), however no induction of CaHsp12p was observed when shifting the temperature from 30°C to 37°C (Figure 2C). Apart from heat shock, CaHsp12p is also induced in osmotic stress such as sodium chloride (NaCl) and sorbitol, oxidative stress such as hydrogen peroxide (H₂O₂), and the heavy metal cadmium (Cd²⁺) (Figure 2C). We noted that even exposure to low doses of NaCl (0.1 M) or H₂O₂ (0.4 mM) resulted in significant induction of CaHsp12p expression (Figure 2C). However, CaHsp12p is slightly increased when grown in high concentrations of sorbitol (0.3 M) (Figure 2C). Expression is only enhanced in cells treated with higher levels of heavy metal Cd²⁺ (0.5 mM), but not in the lower doses (0.1 mM Cd²⁺) (Figure 2C). Similar to CaHsp12p, ScHsp12p is induced by heat shock (from 30°C to either 37°C or 45°C (Figure 2C). Interestingly, unlike CaHsp12p, ScHsp12p was not regulated by H₂O₂ (Figure 2C). Analysis of C. glabrata HSP12 transcript levels revealed an 8-fold induction following heat shock from 37°C to 45°C (Figure 2C). CgHSP12 was 15-fold up-regulated after exposure to 0.3 M NaCl (Figure 2C) but the transcript level was slightly decreased (2-fold) following exposure to 1 mM H₂O₂ (Figure 2C). Finally we show that C. albicans Hsp12p protein is down-regulated by physiological CO₂ and pH (Figure 2C).

C. albicans, S. cerevisiae and C. glabrata Hsp12p is induced by the quorum sensing molecule farnesol

Using qRT-PCR, Davis-Hanna et al. have previously shown that transcription of CaHSP12 was influenced when C. albicans was grown in the presence of the quorum sensing molecule farnesol [24]. Consistent with this work we show that CaHsp12p protein levels sharply increase in response to 100 μM farnesol (Figure 2C). Interestingly, ScHsp12p is also highly induced (Figure 2C), and CgHSP12 is 6-fold increased upon exposure to farnesol (Figure 2C).

Polyene but not azole antifungal agents impact on CaHsp12p expression

Previous work by Coste et al. has shown that the promoter of CaHSP12 contains a cis-acting drug-responsive element (DRE)-like region with four mismatches [26]. Additionally, CaHSP12 was found to be up-regulated in azole-resistant strains [27,42]. Moreover, CaHSP12 is induced when the cells are exposed to fluphenazine, which can also induce multidrug transporter genes [27]. However, there is no direct evidence showing if CaHSP12 is regulated by antifungal drugs. Therefore, we investigated whether CaHsp12p is regulated when the cells were treated with 4 μg ml⁻¹ of the azole drugs fluconazole, ketoconazole, itraconazole, or 2.5 μg ml⁻¹ of the polyene antifungal agent amphotericin B. No significant change of CaHsp12p level was been found when C. albicans was treated with azole antifungal drugs (Figure 2C). Interestingly, CaHsp12p is down-regulated upon exposure to amphotericin B (Figure 2C). C. glabrata HSP12 is also not regulated when the cells were exposed to 4 μg ml⁻¹ fluconazole whereas ScHSP12p is slightly down-regulated in the presence of itraconazole and significantly decreased upon exposure to amphotericin B (Figure 2C).
expression of CaHsp12p was repressed in the efg1 mutant (Figure 3A), suggesting that Efg1p functions as an activator of CaHsp12p. CaHsp12p was also slightly repressed in the tup1 mutant. In contrast, elevated levels of CaHsp12p were observed in the hog1, cyr1, cph1 and, sfl1. The level of CaHsp12p was slightly increased in tpk1 but not tpk2 mutants in unstressed conditions, suggesting that Hog1p, Cyr1p, Cph1p, Sfl1p and Tpk1p but not Tpk2p, repress the production of CaHsp12p (Figure 3A).

Efg1p is required for the expression of CaHsp12p during heat shock and oxidative but not osmotic stress

In order to determine the role of Hog1p and cAMP signaling pathway on the regulation of CaHsp12p in response to stress, expression of CaHsp12p was examined in the hog1, cyr1, cph1, tpk1, tpk2 and efg1 deletion mutants after exposure to heat shock from 37°C to 45°C, 0.3 M NaCl and 1 mM H2O2. Western blots showed that CaHsp12p levels are reduced in the efg1 mutant following heat shock and exposure to NaCl, but not H2O2. This suggested that Efg1p is required for the expression of CaHsp12p under heat shock and oxidative stress, but not to osmotic stress (Figure 3B). These result indicated that there are distinct mechanisms for osmotic stress response and for heat and oxidative stress response in C. albicans. The level of CaHsp12p expression remained high in the hog1, cyr1 and tpk1 deletion mutants exposed to stress (Figure S1). Also, the level of CaHsp12p was not changed between the control strain and the tpk2 deletion mutant under stress (Figure S1).

HSP12 is not essential for growth, stress resistance or virulence

To gain insight into the function of Hsp12p in both C. albicans and C. glabrata we constructed hsp12 null mutants in both species. This required deletion of all four HSP12 alleles in C. albicans, and the single gene in C. glabrata. Determination of the growth rates or cell adhesion of the CaHsp12 and the CgHsp12 deletion mutants did not reveal any differences when compared with their control strains (Figure S2). Furthermore, similar growth on medium supplemented with either osmotic stressors such as sodium chloride, sorbitol; oxidative stressors such as H2O2, menadione;
cell wall and cell membrane stressors such as Congo red, calcofluor white, caffeine and SDS or antifungal drugs such as itraconazole, ketoconazole, fluconazole and amphotericin B did not reveal differences in survival or growth (Figure S3 and S4). The CaHsp12 deletion mutant did not show any difference in germ tube formation when compared with its control strain (Figure S5).

Figure 2. Hsp12p is regulated in response to a wide range of stresses. (A) CaHsp12p is recognized by a polyclonal antibody. The anti-CaHsp12p antibody was tested by using Western blot analysis against protein samples from the C. albicans CAI4 control strain (Ct) and the CaHsp12 null mutant (Dhsp12). The arrow indicates the 13 kDa band of CaHsp12p which is present in CAI4, but absent in the CaHsp12 null mutant. (B) Induction of Hsp12 in C. albicans and in S. cerevisiae during stationary growth. Total protein was extracted at the indicated time points from C. albicans CAI4 at 37°C or S. cerevisiae BY4741 at 30°C. Western blots were probed with anti-Hsp12p antibody and showed a band corresponding to the expected size of 13 kDa. Blots were probed with anti-actin antibody as loading control. Growth curves with sampling time points (open or solid dots) are shown. RDU: relative densitometry units. (C) Hsp12p is regulated in response to diverse conditions. Hsp12 protein level in C. albicans CAI4 or S. cerevisiae BY4741 was assayed using Western blot and a band of the expected size (13 kDa) was detected. H: anti-Hsp12p antibody. A: anti-actin antibody (equal protein loading control). RDU: relative densitometry units. CgHSP12 transcript level was determined by qRT-PCR with total RNA extracted from the Cg2001TU strain. The transcript level was normalized to the Act1 control. The error bars represent the S.D. of triplicate independent reactions. **P value<0.01, * P value>0.3, two-sided unpaired student t-test.

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The results indicate that Hsp12p is not essential for the growth, cell adhesion, filamentation and stress resistance in *C. albicans* or *C. glabrata* under standard laboratory conditions.

In order to study whether CaHsp12p is essential for virulence of *C. albicans*, a virulence test of the CaHsp12p null mutant was carried out in a Toll deficient *Drosophila* line as previously described by us [7]. There was no significant difference in the survival of flies infected with either the CaHsp12 null mutant or its control strain (Figure S6), indicating that deletion of CaHSP12 did not affect the virulence of *C. albicans*.

**Overexpression of CaHSP12 enhances cell aggregation**

To explore the function of CaHsp12 protein further, the gene was expressed under the control of the native *TEF2* promoter generating *C. albicans* HSP12OE. Overexpression was confirmed by using qRT-PCR, showing that the expression of CaHSP12 is increased by 15-fold (Figure 4A). The elevated level of Hsp12p in HSP12OE was also seen in Western blot analysis (Figure S7). Although we observed no alterations in stress resistance, including heat shock, osmotic and oxidative stress (Figure S8), HSP12OE was found to form clumps of cells when grown at pH 7 in liquid medium (Figure 4B). Subsequently, cell-aggregation was quantified in liquid filamentation assays, 100 μM farnesol reduced early-stage germ tube formation to a lesser degree in HSP12OE when compared to the control strain (Figure 4G). Since serum-induced germ tube formation in HSP12OE was not enhanced (Figure 4G), we directly attribute the observed phenotype to a reduced response to farnesol. Overexpression of CaHsp12p specifically enhanced susceptibility to the azole antifungal agents itraconazole, ketoconazole and fluconazole (Figure 4H) but not amphotericin B and rapamycin (Figure S8).

**Discussion**

Although small heat shock proteins are found in most organisms [51] their functions are still poorly understood. Previously, we have shown that expression of the *C. albicans* small heat shock gene, HSP12, is regulated by physiologically levels of CO2 and pH [21]. Interestingly these studies revealed that the *S. cerevisiae* orthologue of *HSP12* is unaffected by the change of pH, suggesting different mechanisms of adaptation between the two yeasts [21].

Here we characterize CaHSP12 from the fungal pathogen *C. albicans* and show that it has both similar and different features when compared with other fungal species. CaHSP12 differs from other fungal species for carrying two different loci of the gene, both of which are transcriptionally expressed. We found that CaHsp12p and ScHsp12p are diversely regulated in response to oxidative stress. *C. albicans* has a greater level of resistance to oxidative stress.
Figure 4. Overexpression of CaHSP12 in C. albicans. (A) qRT-PCR analysis of the CaHSP12 transcripts in HSP12OE. The level of transcripts was normalized to ACT1. The error bars represent the S.D. of triplicate independent reactions. (B) Overexpression of CaHSP12 induced cell clumping. The control CAI4+pFM2 and HSP12OE were grown at pH 7. (C) Overexpression of CaHSP12 promoted cell aggregation which was independent from filamentation. CAI4+pFM2 and HSP12OE were grown at pH 4 for 4 h. Aggregation was then measured. The graphs were plotted by the percentage of cells sedimented against time. Results represent the means of three biological replicates with S.D. *P value < 0.05, versus control strain, two-sided unpaired student t-test. (D) Overexpression of CaHSP12 enhanced cell adhesion at pH 4 or pH 7. HSP12OE and CAI4+pFM2 were grown on the flat-bottomed 96-well polystyrene plates and incubated at 37°C for 24 h. The adherent cells were quantified using the XTT reduction assay. The error bars were calculated from the S.D. of the triplicates. *P value < 0.01, versus control strain, two-sided unpaired student t-test. (E) Overexpression of CaHSP12 promoted filamentation at pH 7. The percentage of the germ tube formation was counted every 30 min. The results presented are the means of three biological replicates with the S.D. **P value < 0.01, *P value < 0.05 versus control strains, two-sided unpaired student t-test. (F) Overexpression of CaHSP12 impacts on farnesol susceptibility. Cells were spotted onto 5% serum YEPD plates supplemented with or without 100 μM farnesol. Scale bar,
Overexpression of CaHSP12 increased the sensitivity of the cells to several azole antifungal drugs. The action of azoles on fungi is mediated by depletion of ergosterol, which results in the alteration of membrane fluidity [60]. In *S. cerevisiae*, S.Hsp12p is known to influence plasma membrane fluidity enhancing the stability of the cell membrane [61]. Overexpression also enhanced cell adhesion. Interestingly the actions of adhesion are mediated by cell wall proteins [1] and S.Hsp12p has been shown to be localized in cell wall [62]. CaHsp12p has 43% homology to the amino-terminal region of S.Hsp12p, thus it is feasible to speculate that CaHsp12p is present in the cell wall and as a heat shock protein it may have a role in protection of cell wall proteins. Localization studies of CaHsp12p in *vivo* in response to stress and during the yeast-to-hyphal transition are required to address this further.

We show the CaHsp12p is significantly regulated under a wide range of stimuli, but is not essential for *C. albicans* to survive in those conditions. This raises the possibility that other proteins with similar functions may compensate for the inactivation of CaHsp12p in the CaHsp12 null mutant. Our overexpression studies point to the potential role of CaHsp12p in protecting the targets of farnesol, the cell membrane and cell wall protection. Therefore, identifying protein partners of CaHsp12p should be of interest and reveal additional information on its biological function.

**Supporting Information**

**Figure S1** Expression of CaHsp12p in *C. albicans* mutant strains. Western blot analysis showing that levels of CaHsp12p remained high in *hgl1*, *cyr1*, *tpk1* mutants and unchanged in *tpk2* mutant when heat shocked from 37°C to 45°C, 0.3 M NaCl or 1 mM H2O2. H: anti-Hsp12p antibody. A: anti-actin antibody (equal protein loading control). RDU: relative densitometry units. (TIF)

**Figure S2** Deletion of HSP12 does not affect growth rate and cell adhesion. (A) No significant change in the growth rates of the Ca_tls2 ([HSP12KO5] and Cg_tls2 ([Cg12KO) null mutants was observed. The overnight cultures were diluted into the OD<sub>600</sub> of 0.1 and incubated at 37°C. The OD<sub>600</sub> of the cells was measured at the indicated time points. The growth curves of strains were plotted in the OD<sub>600</sub> against time. Triplicate biological experiments have been performed. The error bars represent the S.D. of the triplicate independent experiments. (B) The Ca_tls2 and the Cg_tls2 null mutant displayed the same ability of cell adhesion as controls in the XTT reduction assay. The strains were grown on the flat-bottomed 96-well polystyrene plates and incubated at 37°C for 24 h. The adherent cells were quantified using the XTT reduction assay. The results presented are the means of three biological replicates with standard derivation. *P* value &gt;0.05 versus controls, two-sided unpaired student t-test. (TIF)

**Figure S3** CaHSP12 is not essential for *C. albicans* in resistance to stresses and antifungal drugs. Overnight cultures were diluted in YEPD liquid to an OD<sub>600</sub> of 2. For heat shock test, the cells were heated at 55°C for 2 min and 10-fold dilutions of the cells were spotted onto YEPD. For other stress...
tests, the cells at 10-fold dilutions were spotted onto YNB plates containing stress or antifungal agents as indicated. The cultural plates were incubated at 37°C for 24 h. The YNB plates supplemented with 1% chloroform, methanol and DMSO act as control of itraconazole, ketoconazole and fluconazole which were dissolved in chloroform, methanol and DMSO. (TIF)

**Figure S4** Deletion of CgHSP12 did not affect resistance to stress and antifungal drugs. The overnight cultures were diluted in YEPD liquid to an OD_{600} of 2, and heat shock at 55°C for 2 min. The cells at 10-fold dilutions were spotted onto YEPD plates and incubated at 37°C for 24 h. For other stress tests, the cells at 10-fold dilutions were spotted onto YNB plates containing stress or antifungal agents as indicated. The cultural plates were incubated at 37°C for 24 h. The YNB plates supplemented with 1% chloroform, methanol and DMSO act as control of itraconazole, ketoconazole and fluconazole. (TIF)

**Figure S5** Deletion of CgHSP12 does not interfere with filamentation at pH 7 in 5.5% CO_{2}. The CgHsp12 deletion strain and its controls were incubated in YNB minimal medium at pH 7 in 5.5% CO_{2} at 37°C. The cell morphology of the strains was observed by a light microscopy. The percentage of the germ tube formation was counted under the microscopy every 30 min. The germ tube formation of the CgHsp12 null mutant had no significant difference to the controls. Results presented are the means of three biological replicates with standard derivation. *p value>0.1 versus controls, two-sided unpaired student t-test. (TIF)

**Figure S6** Deletion of CgHSP12 does not influence the virulence of *C. albicans* in the Toll mutant fruit fly. 15 flies per experimental group were injected with the *C. albicans* strains. The flies were then incubated at 30°C for 40 h. The numbers of the living flies were counted at the indicated time. The results are calculated from the means of three biological replicates with the standard derivations. *P value >0.1, versus control strains (WT or HSP12C), two-sided unpaired student t-test. (TIF)

**Figure S7** Western blot analysis of the CaHsp12p expression in HSP12OE. CaHsp12p was expressed higher in the HSP12OE when compared to wild-type. The blot was hybridised with the anti-Hsp12p antibody and the anti-actin antibody, served as the control for equal protein loading as described in text S1. (TIF)

**Figure S8** Overexpression of *CaHSP12* does not affect growth under stresses and exposure to antifungal agents. For the heat shock assay, the overnight cultures were diluted to OD_{600} of 2 and shifted to 55°C for 2 min. The 10-fold serial dilutions of the heat shock cells were spotted onto YEPD plates and incubated at 37°C for 24 h. For other stress studies, the overnight cultures at the OD_{600} of 2.0 were diluted 10-fold serially. The dilutions (5 μl) were spotted onto YNB plates supplemented with stress and antifungal agents as indicated. The plates were incubated at 37°C for 24 h. (TIF)

**Figure S9** Overexpression of CaHSP12 promotes cell aggregation at pH 7 in air or 5.5% CO_{2}. The strains were grown at (A) pH 7 in air; (B) pH 7 in 5.5% CO_{2}. Total 1 ml of the culture was settled to the bottom of the cuvettes. The OD_{600} corresponding to the cells at the upper part of the cuvettes was measured at the time points indicated. The graphs were plotted by the percentage of cell sedimented against time. Results represent the means of three biological replicates with standard derivation. *P value <0.05, versus control strain, two-sided unpaired student t-test. (TIF)

**Text S1** Supplemental Materials and Methods. (DOC)

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**Author Contributions**

Conceived and designed the experiments: MSF LD FAM. Performed the experiments: MSF LD. Analyzed the data: MSF LD FAM. Contributed reagents/materials/analysis tools: MSF LD FAM. Wrote the paper: MSF LD FAM.

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