HAC1 is expressed in *Candida parapsilosis*-a human fungal pathogen

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Abstract

Candida parapsilosis an opportunistic pathogen, exist as a human commensal. It is the second most common cause of nosocomial candidemia and is showing increased resistance to the existing drugs. It is postulated that increased resistance is due to active ER stress response pathway. In this report, we have characterized the key molecule of this pathway *HAC1* and found that it is expressed in ER stress conditions. It is susceptible to UPR stress inducer, suggesting perhaps it is functional in this organism. Since the UPR pathway is responsible for fungal pathogenesis we envision further characterization will suggest methods for immunotherapy.

Keywords: *Candida parapsilosis*, *HAC1*, ER stress response, RT-PCR

Main text:

C. albicans is the leading cause of invasive candidiasis. However the epidemiology of yeast infection is rapidly evolving, and non-albicans *Candida* species and other fungi have emerged as a significant opportunistic pathogen. Among the non-albicans yeast, *Candida parapsilosis* is the principal cause of candidiasis, especially in neonates and patients in intensive care units [1]. It harbors in the skin, gastrointestinal tracts and mucous membranes. However, lower immunity due to indiscriminate use of antibiotics or immunosuppressive drugs turns these microbes pathogenic [2]. Among the hospital-acquired blood infections, *C. parapsilosis* is responsible for about 15% of Candida infections. It is the second most commonly detected Candida species in blood cultures in Europe, Canada, and Latin America [3]. It is been postulate that UPR pathway, biofilm formation, hydrolytic enzymes, and adhesion to the skin or mucosal membranes for disease prognosis [4]. *C. parapsilosis* in mild condition, manifest as a red and painful infection of the eyes, mouth or vagina. In severe cases, it infects the internal membranes, peritoneum and causes a systemic shock with a danger of death. In rare cases, it may cause septic arthritis or pneumonia [5]. Multiple antifungal medications had been suggested for *Candida parapsilosis* infections Caspofungin, fluconazole, and Amphotericin [6]. However, it is showing resistance to the existing drugs. So, in order to better understand the resistance mechanism and disease prognosis, we searched for active UPR pathway.

Unfolded protein response pathway (UPR) also known as endoplasmic reticulum (ER) stress response has emerged as one of the key determinants of fungal pathogenesis [7]. This pathway is reported in *Cryptococcus* [8], *C. albicans* [9], *C. glabrata* [10], *Aspergillus* [11] and other filamentous fungi [7]. Most of the nascent and secretory proteins are folded in ER lumen. However, the folding capacity of the ER is hampered by various pathological or physiological cues lead to the accumulation of misfolded or unfolded protein in the ER lumen, causing ER stress. UPR a homeostatic adaptation pathway help in relieving secretory load for pathogens and thus help in successful invasion and disease establishment. UPR helps the fungus to meet the protein secretion and folding demands in the host. Sometimes protein folding exceeds its capacity leading to accumulation of unfolded protein in the ER lumen. The two key molecules of UPR pathways are IRE1 (Inositol-requiring enzyme 1) and HAC1 (Homologous to ATF/Creb1) /HXL1 (HAC1 and XBP1-Like gene 1). Ire1p is an ER transmembrane protein and HAC1/HXL1 is a b-ZIP transcription factor, a downstream transducer. Upon ER stress, Ire1 is activated and cleaves a regulatory intron in HAC1/HXL1 mRNA to remove a translational block. Despite the fact, UPR pathway and fungal pathogenesis are intricately related [7] its existence in *C. parapsilosis* is elusive.

So in order to understand this pathway we have taken related baker's yeast, *Saccharomyces cerevisiae* WT strain (MTCC accession number 170) and *Candida parapsilosis* (NCIM accession number 3323). The cells were grown in standard YEPD (1% yeast extract, 2% peptone, and 2% dextrose) and Synthetic complete (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% dextrose) medium with essential amino acids at 28° C. To study the transcript and whether HAC1 gene is expressed or not, we have isolated the total RNA from log phase yeast cells (OD_{600nm} 0.8). Isolation of RNA is tricky as little information is available regarding the composition of the cell wall of *C. parapsilosis* [12]. Thus, we used a method which is proven to isolate difficult to isolate RNA [13]. The total RNA was further purified and treated with DNase-I NP-84105 RNASure® Mini Kit (Genetix) as per the manufacturer instructions. The purified RNA was quantified in a spectrophotometer and resolved in denaturing formaldehyde gel electrophoresis. The total RNA was resolved in denaturing formaldehyde gel electrophoresis (Fig.2B) and quantified by spectrophotometer. We obtained an A260/A280 ratio of 2.0 and a concentration of 2.05 mg/mL of total RNA. The RT-PCR was performed as per previous method [14] in 20 µL reaction using M-MuLV Reverse Transcriptase (Himedia) using gene-specific reverse primers. The second step PCR amplification was performed using Taq DNA polymerase (Himedia) using forward and reverse primers of *C. parapsilosis* based on annotated clone contig 005569 5'-CTGGTCGACATGGACGTAGATACTACT, 5'-CTGGGATCCTCATACAGAAACGGCCAG respectively. The primers for *S. cerevisiae* 5'-CGCAATCGAACTTGGCTATCCCTACC and 5'-GGGTAGACTGTTTCCCGC correspond to nucleotide sequence +35 to 60 (where +1 denotes the adenine of the start codon AUG) and the nucleotides +604 to 621 respectively.

The dilution spotting was performed by serial dilution method in absence and presence of Dithiothreitol DTT (5, 10, 15 mM) to induce ER stress response [15]. Our results suggest that there is 20 fold reduced growth in 15 mM of DTT stress conditions as compared to control (Fig.1). The RT-PCR results suggest HAC1 transcript is ~ 1.2 Kb band and is expressed in the presence and absence of ER stress inducer DTT (Fig. 2A). To rule out the possibility that the amplified PCR product is derived from RNA only, we have used negative control without reverse transcriptase in the reaction. The result suggests that there is no amplification in Lane 2, 3 and 6, 7 (Fig 2C). As a control we have used *S. cerevisiae* total RNA for RT-PCR analysis, the amplified band is as expected and described previously [16].

Among the non-albicans Candida species, *Candida parapsilosis* has become the second most common opportunistic organism to vulnerable and immunocompromised individuals. The major challenges are increased resistance to the existing drugs. The infection is paradoxically also linked with medical and surgical instruments, medicines, organ transplants and the use of broad-spectrum antibiotics [17,18]. Thus it is imperative to look into the signaling mechanism of disease prophylaxis and therapies. The recent reports suggest that UPR and fungal diseases are linked [19]. Blocking this pathway could be a great strategy to reduce fungal microbiome load. In this report, we have reported functional *HAC1* gene and showed that *C. parapsilosis* is susceptible to UPR stress inducer. Further studies by gene disruption of the particular gene will shed important lights and suggest avenues for immunotherapy.

Acknowledgment: R.S. and M.A.M would like to acknowledge the School of Bioengineering and Biosciences and Division of Sponsored Research at Lovely Professional University for basic infrastructure and financial assistance for performing the task. This work was supported by an intramural grant to M.A.M from Division of Research and Development at Lovely Professional University, Phagwara, India.

Competing interest: The authors declare no conflict of any competing interest.

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Figure legends

Figure 1: *C. parapsilosis* showed dose-dependent growth inhibition. The growth of *Saccharomyces cerevisiae* (S. c.) and *Candida parapsilosis* (C. p.) is tested over different concentration of ER stress inducer Dithiothreitol (DTT) in SC medium.

Figure 2: RT-PCR analysis of the *HAC1* gene. (A) The RT-PCR of HAC1 gene was performed using total RNA isolated from yeast strains using M-MLU reverse transcriptase. The corresponding 1 Kb ladder is shown in Lane 1, Lane 2 and 3 corresponds to *Saccharomyces cerevisiae* (S. c.) and Lane 4 and 5 corresponds to *Candida parapsilosis* (C. p.) HAC1 transcript. The 1.2 Kb band of HAC1 transcript is shown by (*). (B) The large rRNA as a loading control is shown in 25 S and 18 S as an arrowhead. (C) RT-PCR in the absence (-), Lane 2,3 and 6, 7 and presence (+), Lane 4,5 and 8, 9 of *Saccharomyces cerevisiae* (S. c.) and *Candida parapsilosis*(C.p.) respectively.

Figure 1

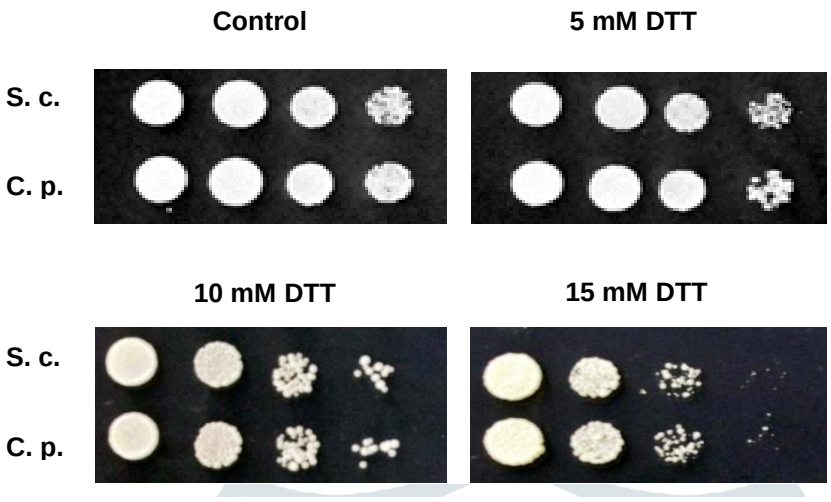

Figure 2