ASSESSMENT OF ENZYMATIC VIRULENCE TRAITS OF *CANDIDA ALBICANS* IN IMMUNOCOMPROMISED CANCER PATIENTS

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ABSTRACT

Background: *Candida albicans* is an opportunistic fungal pathogen commonly found in immunocompromised individuals, including cancer patients. The expression of virulence-associated enzymes plays a crucial role in its pathogenicity. **Objective:** This study aimed to evaluate the enzymatic virulence profiles of *Candida albicans* isolates obtained from cancer patients and compare them to the reference strain ATCC 10231. **Methods:** A total of 46 clinical isolates were assessed for the production of protease, lecithinase, lipase, phospholipase, esterase, hemolysin, coagulase, and DNase using standard plate-based assays. Enzyme activities were recorded and classified as negative, low, moderate, or high. **Results:** Proteolytic activity was commonly expressed among clinical isolates and consistent with the ATCC 10231 reference strain, with only three isolates lacking protease production. Lecithinase and lipase activities were uniformly negative in both clinical and reference strains. In contrast, none of the clinical isolates showed high phospholipase activity; 80.4% were categorized as low producers, and 19.6% exhibited moderate activity. Esterase and hemolysin activities were mostly moderate, comparable to the reference strain. For coagulase, only 34.8% of isolates showed activity at 4 hours, increasing to 63.0% after 24 hours. DNase activity varied, with 17.4% of isolates showing no detectable activity. **Conclusion:** The study reveals conserved expression of some enzymatic virulence factors such as protease, esterase, and hemolysin among *C. albicans* isolates from cancer patients, while phospholipase, coagulase, and DNase activities exhibited strain-specific variability. These findings highlight the heterogeneous expression of virulence determinants in clinical isolates and underscore the need for individualized assessment in high-risk patient populations.

Keywords: *Candida albicans, Virulence Factors, Fungal, Enzymes, Clinical Isolates*

I. INTRODUCTION

Candida albicans is the most prevalent fungal pathogen in humans and is responsible for both superficial and systemic candidiasis. As a commensal organism, it colonizes the oral cavity, gastrointestinal tract, and genitourinary system of healthy individuals. However, in immunocompromised or critically ill patients, it can transition into an opportunistic pathogen, causing infections with significant morbidity and mortality (Calderone & Fonzi, 2001; Mayer et al., 2013). The increasing prevalence of antifungal resistance and the emergence of non-*albicans Candida* species have further complicated clinical management, underscoring the need to better understand the mechanisms underlying fungal virulence (Deorukhkar et al., 2014; Silva et al., 2012).

A key strategy used by *C. albicans* to invade host tissues and establish infection involves the secretion of hydrolytic enzymes. These enzymes degrade host structural

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components such as proteins, lipids, and nucleic acids, facilitating nutrient acquisition and tissue penetration. The three most well-characterized enzyme families are secreted aspartyl proteinases (SAPs), phospholipases, and lipases.

Phospholipases, particularly PLB1 and PLB2, are capable of hydrolyzing phospholipids in host cell membranes, leading to cytolysis and tissue invasion (Ghannoum, 2000; Ibrahim et al., 1995). The presence and activity of phospholipases are commonly assessed using egg yolk agar (EYA), where a precipitation zone around fungal colonies indicates positive enzymatic activity (Price et al., 1982; Samaranayake et al., 1984). Clinical studies have shown a correlation between phospholipase production and increased virulence in systemic candidiasis (Tsang et al., 2007). Lipases (LIP1–LIP10) catalyze the hydrolysis of host triglycerides, contributing to colonization of lipid-rich niches such as the skin and sebaceous glands. Lipase 8 (Lip8), for instance, has been implicated in immune evasion and tissue damage in systemic infections (Staib et al., 2000; Gácser et al., 2007). Beyond these canonical enzymes, *C. albicans* also exhibits several additional enzymatic activities that are considered virulence factors and can be detected using basic biochemical assays.

Hemolysins lyse red blood cells to extract iron from hemoglobin, a vital micronutrient that is limited in the host environment. Hemolysin production is detectable on blood agar and has been linked to increased virulence in both oral and systemic isolates (Manns et al., 1994; Loster et al., 1999; Schaller et al., 2005). Esterases hydrolyze ester bonds in host lipids and are detected using Tween 80 opacity tests. These enzymes may contribute to tissue colonization, particularly in environments rich in lipids such as the oral cavity and skin (Ribeiro et al., 2002). Elevated esterase activity has been noted in strains from diabetic and HIV-positive patients (Tsang et al., 2007; Agwu et al., 2001). Coagulase-like activity, although not widely characterized in *C. albicans*, has been reported in some clinical isolates. It may facilitate the formation of fibrin barriers that shield fungal cells from phagocytes, mimicking a well-known virulence strategy of *Staphylococcus aureus* (Ellepola & Samaranayake, 2001). DNases degrade extracellular DNA, possibly aiding in evasion of neutrophil extracellular traps (NETs). DNase-producing isolates show increased tissue invasion and may suppress host immune responses (Martins et al., 2010; Tamura et al., 2018).

Strain-specific differences in enzyme production have also been documented. Isolates from bloodstream infections, diabetic patients, and HIV-positive individuals often express higher levels of these enzymes compared to those from asymptomatic carriers (Silva et al., 2009; Agwu et al., 2001). Furthermore, high enzyme expression often correlates with poor clinical outcomes, particularly in settings of inadequate antifungal therapy (Tumbarello et al., 2007).

Despite advances in characterizing these virulence factors, several knowledge gaps remain. For instance, the regulation of enzyme expression during host-pathogen interaction, the synergy between different enzymes, and their precise roles in immune evasion are not fully understood. Additionally, while most research has focused on *C. albicans*, emerging non-*albicans Candida* species such as *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*

exhibit distinct enzyme profiles and virulence patterns that require further study (Silva et al., 2012).

In conclusion, the enzymatic virulence factors of *C. albicans* including proteinases, phospholipases, lipases, and auxiliary enzymes like hemolysin, esterase, DNase, and coagulase play integral roles in fungal pathogenicity. Their detection not only provides diagnostic insight into strain virulence potential but also offers targets for novel therapeutic interventions. A deeper understanding of these enzymes in both *C. albicans* and emerging Candida species is critical to address the growing burden of fungal infections in the immunocompromised population. In this study, we investigated the virulence factors of *Candida albicans* isolates obtained from cancer patients, with a particular focus on their enzymatic activity profiles. The findings revealed that protease, lecithinase, lipase, hemolysin, and esterase activities in the clinical isolates were comparable to those of the reference strain *C. albicans* ATCC 10231. In contrast, coagulase, DNase, and phospholipase activities exhibited strain-dependent variability among clinical isolates, demonstrating differing levels of enzymatic expression compared to the standard strain.

II. MATERIALS AND METHODS

2.1. Experimental model

Clinical and reference strains of *Candida albicans* were obtained from stored glycerol stocks and subcultured on Sabouraud Dextrose Agar (SDA) at 4°C. Prior to enzymatic assays, isolates were inoculated into Sabouraud Dextrose Broth (SDB) and incubated at 37°C for 24 hours to ensure active growth. The cultures were then adjusted to a standardized concentration of 1×10^6 CFU/mL using spectrophotometric measurements at OD_{530} to ensure consistency across assays.

2.2. EYA method

Lecithinase, lipase, and protease activities of *Candida albicans* isolates were simultaneously assessed using the egg yolk agar (EYA) method. The medium was prepared by supplementing Egg Yolk Agar Base (Himedia, M808) with 10% sterile egg yolk emulsion (Himedia, FD045). After inoculation a 9 mm loop of standardized fungal suspensions, the plates were incubated at 37°C for 4–5 days. Lecithinase activity was identified by the formation of a white, opaque precipitation zone around the colony, indicating phospholipid hydrolysis. Lipase production was suggested by the appearance of an iridescent sheen or oily layer around colonies. Protease activity, though less distinct on EYA, was indicated by a shallow zone of clearance beneath or around the colony due to protein degradation. These observations allowed for semi-quantitative evaluation of multiple hydrolytic enzymes relevant to *C. albicans* virulence.

Table 1. ***Observation of ezyme activity***

Enzyme	Reaction on EYA
Lecithinase	Opaque, white precipitation zone around the colony due to lecithin hydrolysis
Lipase	Iridescent sheen or oil droplet-like appearance on the colony surface or around it
Protease	Clear zone beneath or around colony (especially on nutrient-based agar) due to protein degradation

2.3. Phospholipase activity

Phospholipase activity was assessed using the egg yolk agar (EYA) plate method with some modifications. The medium consisted of Sabouraud 2% Dextrose Agar supplemented with 0,055% NaCl, 5,5% $CaCl_2$ and 20% sterile egg yolk emulsion. After autoclaving and cooling to 50°C, the yolk was added aseptically. A 9mm loop of standardized fungal suspension were inoculated onto the agar surface and incubated at 37°C for 7 days. Phospholipase activity was determined by the appearance of a precipitation zone surrounding the colony.

2.4. Hemolytic activity

Hemolytic activity was evaluated on SDA plates supplemented with 7% defibrinated sheep blood and 3% glucose. A 9mm loop of standardized fungal suspension were inoculated onto agar surface and incubated at 37°C under 5% CO_2 for 48 to 72 hours. Hemolysis was observed as zones of erythrocyte lysis around the colonies.

2.5. Esterase activity

Esterase activity was determined using a Tween 60 opacity test. The assay medium is SDA supplement with 1% Tween 60 and 0.4% $CaCl_2$. The medium was autoclaved, cooled, and Tween 60 was added aseptically. Isolates were spot-inoculated with a 9 mm loop and incubated at 37°C for 5 days. Esterase activity was detected by the formation of opaque zones around colonies due to the precipitation of calcium salts of fatty acids.

2.6. Coagulase-like activity

Coagulase-like activity was tested by mixing 0.5 mL of fungal suspension with 0.5 mL of commercial human plasma (containing EDTA as anticoagulant) in sterile test tubes. The mixtures were incubated at 37°C and observed at 4, and 24 hours. Positive coagulase activity was indicated by the formation of a clot or gel-like consistency, whereas negative activity showed no visible coagulation.

2.7. DNase activity

DNase activity was assessed using commercial DNase test agar or freshly prepared medium supplemented with toluidine blue as indicator dyes. Isolates were straight-inoculated onto the agar surface and incubated at 37°C for 48 hours. Plates were then examined for zones of clearing or color changes (in dyed media). A distinct halo around colonies indicated DNase production, while the absence of a halo suggested no activity.

All enzymatic assays were performed in triplicate to ensure reproducibility. Measurements of colony diameters and zone sizes were averaged and statistically analyzed using descriptive statistics. Comparative analysis among isolates from different clinical origins was conducted using analysis of variance (ANOVA), and correlations between enzyme activity and clinical features were assessed using Pearson's correlation coefficient or chi-square tests where applicable.

III. RESULTS

In vitro production of hydrolysin enzymes

The enzymatic profile of Candida albicans clinical isolates, including proteolysis, lecithinase, and lipase activity, was largely consistent with that of the reference strain ATCC 10231. Both clinical and reference strains exhibited positive proteolytic activity, while lecithinase and lipase activities were uniformly negative. Notably, only three clinical isolates demonstrated negative

protease activity, indicating that proteolytic enzyme production is a common and conserved trait among the majority of isolates examined.

Although the reference strain Candida albicans ATCC 10231 exhibited a high level of phospholipase activity, none of the clinical isolates demonstrated similar levels. A large proportion of the clinical strains (80.4%) were categorized as low phospholipase producers, while 19.6% exhibited moderate activity. In contrast, esterase production among clinical isolates was comparable to that of the ATCC 10231 strain, with the majority being classified as moderate producers; only 2 isolates (4.3%) exhibited high esterase activity. A similar trend was observed for hemolysin production, where most isolates demonstrated moderate activity, and only 7 strains (15.2%) showed low hemolysin activity (Table 2).

Table 2. ***Number and percentage of*** **C. albicans** ***strains displaying different levels of virulence factors*** **in vitro**

Virulence factor n=46	In vitro expression level		
	High[a] (%)	Medium[b] (%)	Low[c] (%)
Phospholipase (Pz)	0 (0)	9 (19.6)	37 (80.4)
Esterase (Ez)	2 (4.3)	44 (95.7)	0 (0)
Hemolysin (Hz)	0 (0)	39 (84.8)	7 (15.2)

a. Pz, Ez, Hz value ≤ 0.59

b. Pz, Ez, Hz value 0.6 – 0.79

c. Pz, Ez, Hz value 0.8 – 1

While the reference strain Candida albicans ATCC 10231 exhibited positive activity for both coagulase and DNase, clinical isolates demonstrated variable enzyme expression. At 4 hours of incubation, only 34.8% of the clinical strains showed coagulase activity, which increased to 63.0% after 24 hours. In terms of DNase production, 17.4% of the isolates showed no detectable activity, indicating heterogeneity in virulence factor expression among the clinical strains (Table 3).

Table 3. ***Number and percentage of*** **C. albicans** ***strains dislaying coagulase (at 4h and 24h) and DNase*** **in vitro**

Virulence factor (n=46)	*In vitro* expression level	
	Positive (%)	Negative (%)
Coagulase 4h	16 (34.8)	30 (65.2)
Coagulase 24h	29 (63)	17 (37)
DNase	38 (82.6)	8 (17.4)

Relationships between different *in vitro* virulence factors

No relationship was found among the virulence factors (phosphalipase, esterase and hemolysin) as there were low Pearson correlation coefficient (Table 4).

Table 4. ***Pearson correlation coefficient for*** **in vitro** ***virulence factor measurements of*** **Candida albicans** ***isolates.***

	Phospholipase	Esterase	Hemolysin
Phospholipase	1		
Esterase	0.1934	1	
Hemolysin	-0.1533	-0.1966	1

Picture 1. ***Example of phosphalipase, esterase and hemolysin production***

IV. DISCUSSION

The observed low phospholipase activity among the majority of *Candida albicans* isolates from cancer patients suggests a potential shift in enzymatic expression associated with host immunosuppression or antifungal exposure. While the reference strain ATCC 10231 exhibited strong phospholipase production, the absence of high-level activity in clinical strains may indicate adaptive modulation in response to the altered microenvironment of immunocompromised hosts. Previous studies have reported that phospholipase activity is associated with tissue invasion and bloodstream dissemination (Ghannoum, 2000; Ibrahim et al., 1995), yet its downregulation in cancer patients could reflect reduced selection pressure for aggressive invasion due to host vulnerability.

In contrast, the esterase activity profiles of clinical isolates were largely consistent with the standard strain, with most strains showing moderate production. Esterases play a role in the degradation of lipid substrates, facilitating colonization of epithelial surfaces and skin, particularly in areas rich in sebaceous secretions (Ribeiro et al., 2002). The stable expression of this enzyme across isolates may suggest that esterase contributes to mucosal persistence rather than deep tissue invasion, aligning with the frequent occurrence of oral candidiasis in cancer patients undergoing chemotherapy.

The hemolysin activity results further support the role of medium-level enzyme production in facilitating iron acquisition without eliciting strong immune responses. Hemolysins enable *C. albicans* to lyse red blood cells and scavenge iron, a nutrient that is often limited in the host environment (Manns et al., 1994). The predominance of moderate hemolysin activity may represent a balance between nutrient acquisition and immune evasion. Notably, only a small proportion of isolates exhibited low activity, indicating that hemolysin production remains a relatively conserved virulence trait even in cancer-associated isolates.

Collectively, these findings highlight the variability in virulence enzyme expression among *C. albicans* strains isolated from immunocompromised individuals. The reduced phospholipase activity, alongside preserved esterase and hemolysin production, suggests that certain enzymatic traits may be selectively downregulated or retained depending on the host context. This aligns with previous research showing that virulence factor expression in *C. albicans* is dynamic and responsive to environmental and host-related factors (Naglik et al., 2003;

Silva et al., 2009). Understanding these adaptations may provide insights into pathogenesis in vulnerable populations and inform targeted therapeutic strategies.

The observed variability in coagulase and DNase activity among *C. albicans* isolates from cancer patients, compared to the consistent expression in the reference strain ATCC 10231, highlights strain-specific regulation of virulence factors. The increase in coagulase positivity from 34.8% at 4 hours to 63.0% at 24 hours suggests time-dependent enzyme induction, possibly influenced by host conditions such as altered coagulation factors in immunocompromised individuals (Ellepola & Samaranayake, 2001).

Coagulase production, though uncommon in fungi, may aid in immune evasion by promoting fibrin encapsulation, similar to mechanisms described in *Staphylococcus aureus* (Agwu et al., 2001). Meanwhile, the absence of DNase activity in 17.4% of clinical isolates suggests variability in the ability to degrade neutrophil extracellular traps (NETs), a known fungal evasion strategy (Martins et al., 2010; Tamura et al., 2018). These findings reinforce the idea that virulence traits in *C. albicans* are dynamically regulated and may differ depending on host status and infection environment.

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