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The Main Virulence Factors of *Cryptococcus neoformans*, A Review Article.

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ABSTRACT

Cryptococcus neoformans, a capsule encased pathogenic yeast, causes cryptococcal meningoencephalitis in human, particularly among immunocompromised individuals. *Cryptococcus neoformans* is wide spread across different environmental niches, it can be found in soil, animals, poultry excreta and even on trees. The studies on this fungus are limited, in this review we highlighted the most important virulence factors of this zoonotic pathogen. Numerous virulence factors make this fungus an interesting pathogen. These factors include capsule, melanin, and extracellular enzymes. *C. neoformans* is characterized by having many virulence factors that play obvious roles in establishing host infection or surviving in harsh environmental conditions. Among these factors, the polysaccharide capsule is out as it provides protection to the yeast cells against desiccation, oxidation, and the host's immune system. Outstandingly, this fungus produced melanin which may protect the yeast from UV light, oxidative stress, and antifungal damage. Cryptococcal cells may release extracellular enzymes such as phospholipase and urease that strengthen the cell wall and improve fungal invasion. After all, some of these factors are unique to this pathogen and shared with other pathogens, collectively, these are an important armament to overcome hostile environment, evade host immune system, establishing host infection and resist antifungal treatment.

KEYWORD: Capsule; *Cryptococcus neoformans;* melanin; urease; virulence factor.

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INTRODUCTION

Fungal meningoencephalitis is an infectious disease that poses a serious threat to life and has been a substantial public health concern for several years, particularly in countries with low resources [1]. *C. neoformans*, a pathogenic yeast with a capsule, has been identified as the main causative agent of this disease. It has notably affected several immunocompromised groups, particularly patients diagnosed with AIDS (Acquired Immunity Deficiency Syndrome) [2]. *C. neoformans* is naturally present in several environmental sources, such as avian excreta, plants and soil habitats [3]. In addition, a variety of animals, including felines, koalas and even marine mammals like dolphins have been reported to have infection [4,

5]. Also, *C. neoformans* have been documented as etiological agents of mastitis both in sheep and goats as well [6].

Cryptococcosis can develop when spores or yeasts from the environment get inhaled by the host and not removed by the immune system. In the case of an individual with compromised immune function who loses the ability to effectively eliminate the fungal pathogen, there is a potential for the pathogen to spread throughout the central nervous system, leading to severe and potentially fatal meningitis [7]. Several virulence factors are produced by this fungus, resulting in its pathogenicity. capsule, melanin and extracellular enzymes are all examples of these factors [8,9]. Virulence characteristics of the fungus are affected by environmental conditions, allowing *C. neoformans* to successfully adapt to variable environments [10].

The polysaccharide (PS) capsule can provide a protective shield for *C. neoformans* against phagocytosis, which is a process done by specialized immune cells called macrophages to eliminate intruding cells [11], [12]. Melanin synthesis could protect against stresses such as UV radiation, oxidative stress, and antifungal drugs [13,14]. Also, Cryptococcal cells secrete a wide spectrum of enzymes like proteinases, phospholipases and urease that promote cell wall integrity, destabilize host cell membranes, and increase fungal invasiveness once infected [15].

VIRULENCE FACTORS

Capsular Polysaccharide

The main factor contributing to the virulence of *Cryptococcus neoformans* is its capsule, which plays a crucial role in the biology of this fungus [11]. *C. neoformans* develops a polysaccharide capsule to protect itself from being ingested by host phagocytes. It has been reported that, without the presence of specific antibody or complement opsonization, encapsulated *C. neoformans* will not be ingested by immune cells. Immune signaling for antibody production is inhibited by the capsule, and complement proteins are depleted, both of which lead to reduced opsonization and phagocytosis [11]. Furthermore, the cryptococcal capsule serves as a shield against oxidative stressors, and an augmentation in the size of the capsule leads to enhanced protection [16]. Additionally, the capsule could be used as an element of protection against amoeba, which are natural predators of environmental yeasts [17]. This claim is substantiated by empirical findings indicating that engagements with amoeba lead to an increase in the size of capsules [18]. Moreover, if the yeast is engulfed by phagocytes, it has been demonstrated that the PS capsule provides protection against oxidative stress, which is a crucial factor in the degradation of phagolysosomes [19].

This capsule is the primary factor that determines the virulence of *C. neoformans*, accounting for about 25% of the overall virulence. This estimation was made using multivariate linear regression techniques to evaluate the impact of various virulence factors [20]. It has been observed that non-encapsulated mutants of *C. neoformans* are not capable of causing disease [21,22]. The polysaccharide capsule composes of large macromolecules that are cross-linked together to form a matrix. This matrix gradually reduces in density, porosity, and stiffness as it expands outside from the cell wall. This polysaccharide consists of one primary component and two secondary components. Glucuronoxylomannan (GXM) constitutes more than 90% of the capsule and is composed of a mannose backbone with xylose and glucuronic acid substitutions at specific positions [23,24].

The capsule development is aided by several environmental factors, including CO_2 concentration, temperature, pH, and iron development. Interestingly, it has been noticed that microenvironment like human lung or brain has a huge effect on the capsule size, researchers have found that the capsule size is bigger in the lung, while it is smaller when it infects the human brain [25,27]. Also, the temperature could affect the capsule size, it has been determined to be largest at 37° C after investigating the effects of temperature on capsule growth. Capsule size and infection

result were both strain-dependently regulated by the cell [28]. Moreover, the cloning and sequencing of four capsule-associated genes (CAP64, CAP60, CAP59, and CAP10) revealed their centrality to capsule production [29]. Some indications suggest that the growth of capsules includes the incorporation of older and newer subunits, with the newer subunit being located closer to the outer surface of the capsule [30]. When cryptococcal cells are grown in alkaline environments, it promotes the synthesis of the capsule. This indicates that the ionization states of the acidic side groups of the polysaccharide subunits may play a role in this process [27]. The size of the capsule is similarly controlled by the cell cycle [31] and is greater in cells cultivated in medium with a slower rate of growth [27].

The presence of the capsule as a virulence factor indicates that disrupting polysaccharide synthesis could be an interesting approach against cryptococcal infections. Regarding this matter, the discovery that monoclonal antibodies (mAbs) to the capsule gradually break down the PS implies that part of their protective roles may include direct impacts on the capsule [32]. Cells treated with Amphotericin B exhibited a smaller capsule, indicating that most of its antifungal actions in living organisms may be attributed to increasing the susceptibility of cryptococcal cells to host defense systems [33]. Cryptococcal cells with enlarged capsules exhibited resistance to amphotericin B, whereas the acapsular mutant demonstrated greater susceptibility [16]. The polysaccharide capsule is thought to inhibit the absorption of this large antifungal compound. In the same way, the existence of the capsule provided protection for *C. neoformans* against inhibitors that hydrolyze glycolipids [34]. Curiously, the reverse outcome was noted when fluconazole was used. In this case, acapsular mutants showed greater resistance compared to the encapsulated wildtype [16]. The suggested explanation for this atypical outcome is attributed to the hydrophilic nature of fluconazole, which facilitates its absorption through the hydrophilic polysaccharide capsule. However, the expansion of the capsule was also linked to an increase in resistance to fluconazole [35].

Melanin

Melanin is a polymer which is formed from catecholamine oxidation, that accumulates within cell wall of *C. neoformans*. Melanin helps defend the cell from damaging free radicals made by the immune system's cells and makes the cell membrane less permeable. *C. neoformans* cell wall is coated with melanin, which helps the fungus to survive. In addition, forming melanin throughout its cell wall give a formidable protection against host immune defenses, the pathogenic fungus can have this response activated by stress, temperature, or heavy metal exposure. Furthermore, once within the host, macrophages developed a bursts of reactive oxygen species and reactive nitrogen species into the phagosomes, creating a highly oxidative environment that is lethal to most ingested organisms. Studies have shown that moderate environmental doses of RNS stimulate melanin synthesis whereas oxidative stress decreases it. Also, melanin production reduces at 37Ċ [36]. *C. neoformans* synthesizes melanin by the action of laccases that governed by the expression of two distinctive genes (*Lac*1 and *Lac*2) genes, when exposed to exogenous biphenolic substances, such as L-dopa. After expression, laccase enzymes are packaged within secretory vesicles and subsequently transported to the cell wall, where they are anchored by chitin. Alternatively, laccases can be released into the extracellular environment [14].

The melanin composition exhibited a significant association with variations in temperature and pH. Specifically, the optimal conditions for melanin formation were seen at a pH of 8.5 and a temperature of 30 °C. In addition, melanin synthesis serves to shield the fungal cells against oxidative damage caused by hydrogen peroxide. Variations in both temperature and pH have a significant impact on melanin formation in *C. neoformans*, and this is directly related to the

survival of the fungus. Given the limited number of antifungal treatments available and the increasing resistance of cryptococcal infections, it would be beneficial to study how environmental variables affect the process of melanization and survival by *C. neoformans* [37].

Phospholipase

Phospholipases refer to a class of extracellular enzymes responsible for the degradation the ester bonds of phospholipids within the cell membrane, generating fatty acids that can be used as a possible energy source by *C. neoformans* [15,38]. Phospholipases are categorized into four primary types: phospholipase A, also referred to as (PLA1 and PLA2), phospholipase B (PLB), phospholipase C (PLC), and phospholipase D (PLD). This classification is based on their ability to break the ester bond within a phospholipid molecule [39].

Phospholipase B1 (PLB1) was extensively studied and is recognized as a prominent virulence component of *C. neoformans* [40]. The reduction of PLB1 expression resulted in a considerable decrease in fungal loads during lung infection caused by *C. neoformans* [41]. Furthermore, it has been established that PLB1 plays a crucial role in the extrapulmonary dissemination of *C. neoformans* [42], [43], as well as, in the transmission of the yeast through the blood-brain barrier [44], [45]. Significantly, the presence of *PLB*1 is essential for the liberation of arachidonic acid from phospholipids and the synthesis of cryptococcal eicosanoids. These eicosanoids play a crucial role in suppressing the activities of macrophages in vitro and through pulmonary infection [43]. Consistent with this discovery, recent research has indicated that *PLB*1 facilitates the development of *C. neoformans* in macrophages and hinders the elimination of this fungus in the phagosome [46].

Urease

A significant virulence component of *C. neoformans* is the extracellular enzyme urease [47]. *C. neoformans* produces urease, which catalysis urea into ammonia and carbamate [48]. The *URE*1 gene in *C. neoformans* is responsible for encoding the urease apoenzyme. This gene has been subject to deletion and subsequent investigation to better understand its involvement in virulence [47,49].

This enzyme is utilized as a valuable screening test to differentiate *C. neoformans* from *Candida* species in clinical isolates [50], [51]. In addition, this enzyme facilitates the yeast's ability to migrate out the respiratory tract and cross the blood-brain barrier. This is achieved through two different processes: first, an increase in the expression of adhesins on the endothelial cells or second, toxicity directly on the tight junctions of the brain-blood barrier. These findings suggest that URE1 plays a role in central nervous system invasion. However, once the organism has entered the brain, URE1 is not required for their development [49,52]. Due to the ability of *C. neoformans* to utilize urea as its main nitrogen source, *C. neoformans* is thought to benefit from this trait in order to survive and grow in pigeon guano. Previous studies failed to recognize the significance of urea as a vital product of essential internal metabolic processes, such as the urea and purine catabolic cycles, due to the researchers' limited emphasis on external sources of urea [53].

According to a number of studies [49, 54,55], activity of urease facilitates the invasion of cryptococci into the brain. Additionally, an infection in urease-producing *C. neoformans* is linked with augmented eosinophil inflow, greater levels of IL-4, and IL-13, IgE, and, conversely, stimulation of macrophages, showing that activity of urease enhances Th2 immune responses [56].

Surprisingly, a strain of *C. neoformans* was detected with inhibited synthesis of urease has been recorded. The strain was isolated from AIDS patients who had severe cryptococcosis [57]. This particular isolate is the first documented case of a strain of *C. neoformans* which lacks the ability of producing urease [58]. Reports indicate that

urease-positive isolates possess the capacity to develop acute pneumonitis, which could contribute to the animal's mortality prior to the occurrence of significant brain injury. In contrast, urease-negative strains may lack the ability to induce pneumonitis. Nevertheless, *C. neoformans* obtained the chance to disseminate to the brain and trigger meningoencephalitis, causing a significant accumulation in the spinal fluid and finally terminating in death. This will aid in verifying the idea that the strain lacking urease exhibits less pathogenicity in the pulmonary system [47].

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AUTHORS' CONTRIBUTIONS

Collecting information, references and writing of draft version of the article was performed by Sarah Hayder Jabbar, while setting the general concepts, editing, and writing the final version of the article was performed by Ali Hadi Abbas.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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