# ISOLATION OF DNA AND THE ANALYSIS OF MATING TYPES BASED ON GENETIC AND ENVIROMENTAL INFLUENCE IN *CRYPTOCOCCUS NEOFORMANS*

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**BENIN CITY.**

**SEPTEMBER, 2023**

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF B. SC. (HONOUR) IN PLANT BIOLOGY AND BIOTECHNOLOGY**

**UNIVERSITY OF BENIN**

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# CERTIFICATION

The undersigned hereby certify that this work was carried out by Blessing Oluwadamilola EJOGBAMU in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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**Head of Department**

# DEDICATION

This work is dedicated to God Almighty for His ever sufficient grace, infinite mercies and eternal love throughout the course of my studies and research project. Oh, what a father and friend I have in Him. Thank you Olugbeja (God my defender).

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# ABSTRACT

*Cryptococcus neoformans* is an encapsulated yeast that can cause fatal infections in normal and immunocompromised patients. *Cryptococcus neoformans* var. *neoformans* is found mainly in temperate climates and is often isolated from pigeon droppings. It is the causative agent of Cryptococcosis and is believed to arise after inhalation of yeast cells or basidiospores from environmental sources. For the purpose of this research, two strains of *Cryptococcus neoformans;* one clinical FBC and one environmental NBM5 were selected and confirmed using urease medium, based on their melanin production on Niger seed agar and ability to grow at 37$^{o}$C. An antifungal susceptibility test was carried out on the clinical and the environmental isolates using Fluconazole as the antibiotic agent. Fluconazole is an antifungal therapeutic agent used in the treatment of Cryptococcosis. Responses of both isolates to Fluconazole showed similar results. Both isolates were also subjected to environmental stressors using sodium nitrate ($NaNO_2$) to induce nitrosative stress and hydrogen peroxide ($H_2O_2$) to induce oxidative stress. The response of both isolates to this environmental stressors were similar. The selected isolates; clinical isolates FBC and environmental isolates NBM5 were determined to be of the same mating types based on their responses to antifungal susceptibility test and environmental stressors. DNA was isolated from both strains for detailed genetic analysis in the near future. This study provides new insights into how the mating type can greatly influence responses of *Cryptococcus neoformans* to genetic and environmental factors. Thus, serve as a possible instrument for further study of the genetics of *C. neoformans.*

# CHAPTER ONE

# 1.0 INTRODUCTION

*Cryptococcus neoformans* is an encapsulated yeast that can cause fatal infections in normal and immunocompromised patients. *Cryptococcus neoformans* is a basidiomycete that grows as a haploid yeast. Under favourable conditions, cells of opposite mating types fuse to form a teleomorph that is named within the genus Filobasidiella. The yeast typically occupies a saprophytic niche and only rarely infects human and animals. *Cryptococcus neoformans* var. *neoformans* is found mainly in temperate climates and is often isolated from pigeon droppings (Kozel, 1995). *Cryptococcus neoformans* var. *gattii* is found mainly in tropical and subtropical climates, and the tree Eucalyptus camaldulensis is probably one of its environmental niches. Cryptococcosis is believed to arise after inhalation of yeast cells or basidiospores from environmental sources. The organism is free-living and associated with a variety of hosts (Olszewski and Zhang *et al.,* 2010). The main route of infection is through the inhalation of spores from the environment. In humans, *C. neoformans* infection is transmitted by inhalation of basidiospore aerosols. It can invade the central nervous system and cause meningoencephalitis (Velagapudi *et al.,* 2009). Although *C. neoformans* can infect healthy individuals, cryptococcosis is most frequently found in immunocompromised individuals, and although various types of immunosuppression can lead to infection, AIDS has the greatest risk factor (Casadevall and Perfect 1998). However, no specific cellular immunodeficiency predisposing to cryptococcosis is known (Kozel, 1995).

*Cryptococcus neoformans* can be subdivided into four serotypes, A, B, C, and D (Evans and Kessel, 1951; Evans, 1949, 1950; Wilson du *et al,* 1968), with a fifth class consisting of serotype AD occasionally being observed (Cogliati *et al*., 2001; Lengeler *et al.,* 2001). Two

morphologically distinct teleomorphs, Filobasidiella bacillospora and neoformans, have been found to correspond to both varieties. Due to the distinct molecular differences between the two kinds, *Cryptococcus gatti* and *Cryptococcus neoformans* have been designated as separate species (Boekhout *et al*., 2001). The Filobasidiella Clade of the order Tremellales, which also includes non-pathogenic saprophytic parasites associated with decaying flora, grasses, and soil, is home to the two pathogenic parasites found inside the Cryptococcus species complex. The species *C. neoformans* has been divided into two varieties, var. neoformans (serotypes D and AD) and var. grubii (serotype A). According to Kwon-Chung and Varma (2006), *C. neoformans* and *C. gattii* are non-infectious environmental pathogens that differ in important genetic, physiological, ecological and pathological features. Within each species, genetically distinct subgroups that can be classified as potential species share 89–92% nucleotide identity, but can be distinguished morphologically (Bovers *et al*., 2008b). . Serotype A has a wide geographical distribution in clinical and environmental isolates, unlike serotypes B and C, which are mainly restricted to tropical and subtropical regions (Casadevall and Perfect, 1998). However, the ecological niche of this species has evolved after being discovered in temperate regions. Europe has reported the majority of serotype D cases.

## 1.1 MATING TYPES

*Cryptococcus neoformans* has a bipolar mating system, which is classified into two mating types: MATα and MATa. This system is determined by the presence of specific genes located at the MAT locus. The MATα strain carries the gene encoding the α mating factor, while the MATa strain carries the gene encoding the a mating factor. The presence of both mating types in a population is required for sexual reproduction and the creation of genetic diversity (Nielsen *et al*., 2003; Lin *et al*., 2005). Sexual reproduction in *Cryptococcus neoformans* occurs through a

process known as bipolar mating, in which strains of MATα and MATa combine to form sexual crosses. During mating, α mating factor of MATα cells interacts with a mating factor receptor on MATa cells, resulting in the fusion of the two strains and the formation of dikaryotic mycelium. This dikaryotic stage allows the exchange and recombination of genetic material, leading to the generation of spores and the generation of new genetic variants (Kwon-Chung and Bennett, 1978). Mating types in *Cryptococcus neoformans* exhibit a worldwide distribution but with regional variations. MATα mating type is more common worldwide, while MATa mating is less common. Studies have shown that the distribution of mating type could vary across geographic regions, leading to variations in genetic diversity of *Cryptococcus neoformans* populations. These genetic diversity differences have implications for virulence, antifungal susceptibility, and clinical outcomes (Litvintseva *et al*., 2006; Chen *et al*., 2015). Environmental factors play an important role in shaping the distribution of mating types in *Cryptococcus neoformans*. Specific environmental conditions, such as bird droppings or decaying plant matter, have been observed to favor the proliferation of certain mating types. Mating types in *Cryptococcus neoformans* are associated with differences in clinical features, including disease presentation, severity, and outcome. Studies have shown that certain mating types can often be associated with specific clinical manifestations, such as pulmonary or central nervous system infections. Understanding the relationship between mating types and clinical phenotypes can aid in the development of targeted therapies and clinical management strategies (Kwon-Chung and Bennett, 1984; Bovers *et al*., 2006). Studies have also investigated the association between mating types and antifungal susceptibility in *Cryptococcus neoformans*. Variations in antifungal susceptibility profiles have been reported between different mating types, suggesting a potential correlation between mating

type and resistance. This knowledge is essential to guide antifungal treatment strategies and optimize patient care (Zhang *et al.,* 2022).

## 1.2 MATING TYPE LOCUS

The mating type locus in *Cryptococcus neoformans* is commonly known as the MAT locus and consists of two alleles, MATα and MATa. Each allele contains a unique set of genes responsible for regulating mating. The MATα locus is characterized by genes encoding α mating factor and homeodomain proteins, while the MATa locus contains genes encoding 'a' mating factor and pheromone receptors. These genes play important roles in cell-to-cell mediating, cell recognition and fusion during sexual reproduction (Kwon-Chung and Bennett, 1993; Lin *et al*., 2005). The evolution of the mating locus in *Cryptococcus neoformans* involves a variety of mechanisms, including gene conversion, recombination, and horizontal gene transfer. These processes contribute to allele diversity and maintenance of a balanced ratio between MATα and MATa strains in natural populations. Studies have shown that the MAT locus in *Cryptococcus neoformans* exhibits a bipolar mating system, which has been conserved throughout its evolutionary history (Lin *et al*., 2007). The presence of two distinct mating types, MATα and MATa, allows *Cryptococcus neoformans* to reproduce sexually through a process known as bipolar mating. When MATα and MATa cells come into contact with each other, they release pheromones that activate signaling pathways and induce cell fusion. This fusion leads to the formation of a diploid hyphae, in which two nuclei of different mating types coexist. The dikaryotic stage allows genetic recombination and spore production, promoting genetic diversity and adaptation (Metin et al., 2010). The mating type locus in *Cryptococcus neoformans* is of clinical significance. Certain mating types have been associated with variations in virulence, pathogenicity, and susceptibility to antifungal agents. Studies have found that specific

combinations of mating types may confer a higher risk of developing cryptococcal disease or affect clinical outcomes and response to antifungal treatment. Understanding the relationship between mating types and clinical phenotypes can help improve diagnostic strategies and develop targeted therapies (Litvintseva *et al*., 2003; Byrnes *et al*., 2009). Environmental factors, such as nutrient availability and temperature, can influence the distribution and frequency of mating patterns in *Cryptococcus neoformans* populations. Some certain environmental conditions have been observed to favor the growth and proliferation of specific mating types, leading to variation in their abundance. This environmental selection may contribute to the adaptation and survival of the fungus in different ecological niches (Lin *et al*., 2007).

## **1.3** VIRULENCE FACTOR: PATHOGENICITY

Virulence factors are defined as molecular mechanisms that allow yeast to survive in the host and cause disease. The pathogenesis of cryptococcal disease is multifactorial and certainly involves the combined action of multiple virulence factors. Unlike the potent toxins produced by many bacterial pathogens, *C. neoformans* does not appear to produce toxic products that clearly contribute to the signs and symptoms of cryptococcosis. The contribution of most known virulence factors to the disease process is subtle and in many cases is associated with enhanced survival of yeast in the host (Kozel, 1995).

### 1.3.1 Polysaccharide Capsule

The polysaccharide capsule of *Cryptococcus neoformans* is a complex structure consisting mainly of glucuronoxylomannan (GXM) and mannoproteins (MPs). GXM, the predominant component, is made up of repeating units of glucuronic acid, xylose, mannose, and other sugar residues. The structure of the capsule varies in size, thickness and density, influencing its impact on fungal pathogenicity (Kwon-Chung and Rhodes, 1986). The polysaccharide capsule plays an

important role in evading the host immune system. The capsule acts as a physical barrier impeding the recognition and phagocytosis of *Cryptococcus neoformans* by host immune cells, such as macrophages and neutrophils. The large size and negative charge of the capsule limit the interaction between immune cells, preventing effective fungal elimination (Zaragoza *et al*., 2009). The polysaccharide capsule exhibits anti-phagocytic properties, preventing the engulfment and destruction of fungal cells by phagocytosis. The capsule interferes with opsoninization, inhibits opsonin deposition and fungal recognition by complement receptors on phagocytes. In addition, the capsule can alter phagosome-lysosome fusion, allowing the fungus to persist in the host cell (Zaragoza *et al*., 2009). *Cryptococcus neoformans* polysaccharide capsules can modulate host immune responses. The GXM component of the capsule is able to bind to and activate complement proteins, resulting in the release of proinflammatory cytokines and the recruitment of immune cells. However, excessive activation of the immune system can also lead to inflammation and harmful tissue damage (Vecchiarelli *et al*., 2013). The polysaccharide capsule possesses antioxidant properties, protecting Cryptococcus neoformans against oxidative stress generated by host immune cells. The capsule can scavenge reactive oxygen species (ROS) and inhibit the production of ROS by immune cells, thereby promoting fungal survival and persistence within the host (Martinez and Casadevall, 2006). The production and regulation of the polysaccharide capsule in *Cryptococcus neoformans* are complex processes influenced by various factors. Environmental cues, such as carbon and nitrogen availability, pH, temperature, and oxygen levels, play a role in modulating capsule synthesis. Genetic factors, including transcriptional regulators and signaling pathways, also regulate capsule formation (Bose *et al*., 2013)

### 1.3.2 Melanin production

*Cryptococcus neoformans* produces two types of melanin, eumelanin and dihydroxyphenylalanine (DOPA). Melanin biosynthesis is mediated by enzymes such as laccase and polyketide synthase (PKS) encoded by the Lac1 and PKS1 genes, respectively (Casadevall *et al*., 2000). These enzymes catalyze the conversion of L-DOPA to melanin through a complex biochemical pathway. Melanin acts as a powerful antioxidant, protecting *Cryptococcus neoformans* from oxidative stress induced by host immune cells (Jacobson, 2000). It scavenges free radicals and reactive oxygen species, prevents damage to fungal cells, and promotes survival in the host environment. Melanin contributes to *Cryptococcus neoformans* resistance to phagocytosis by host immune cells. It inhibits the binding of complement components and opsonins to the fungal cell surface, reducin recognition and uptake by phagocytes (Nosanchuk and Casadevall, 2003). In addition, melanin interferes with the production of inflammatory cytokines, reduces the immune response and facilitates the survival of fungi. Melanin plays a role in *Cryptococcus neoformans* resistance to various antifungal agents, including azoles and amphotericin B (Nosanchuk and Casadevall, 2006). Melanin binds to and sequesters antifungal drugs, preventing their effective action and contributing to treatment failure and the emergence of resistant strains. Melanin improves viability and dissemination of *Cryptococcus neoformans* in the host. It confers resistance to harsh environmental conditions, such as ultraviolet (UV) radiation and desiccation, allowing the fungus to persist in diverse ecological niches (Jacobson, 2000). Melanin also facilitates adhesion to host tissues and promotes the formation of biofilms, aiding in the establishment and dissemination of infection.

### 1.3.3 Phospholipase and Proteinase Enzymes

*Cryptococcus neoformans* produces phospholipase enzymes, including phospholipase B (PLB1), phospholipase C (PLC1) and lysophospholipase (LYSOPL), which are involved in the

breakdown of host cell membranes and lipid components. These enzymes facilitate the entry and dispersal of fungi within the host (Chen *et al*., 1997). The enzyme phospholipase contributes to the virulence of *Cryptococcus neoformans* through multiple mechanisms. First, these enzymes can degrade phospholipids present in host cell membranes, leading to disruption of host membrane integrity and facilitating the release of nutrients necessary for growth and survival of the fungi (Chayakulkeeree *et al*., 2011). The enzyme phospholipase interferes with host immune responses by degrading immune cell-derived phospholipids. This disruption affects the integrity and function of immune cell membranes, reducing their ability to effectively recognize and eliminate fungi (Chayakulkeeree *et al*., 2011). *Cryptococcus neoformans* also produces proteinase enzymes, including serine proteases and aspartyl proteases, which play important roles in fungal pathogenesis (Almeida *et al*., 2015). Proteinase enzymes produced by *Cryptococcus neoformans* contribute to virulence through several mechanisms. First, these enzymes can degrade components of the host's extracellular matrix, such as collagen and elastin, facilitating tissue invasion and dissemination of the fungus (Alanio *et al* 2011). In addition, proteinase enzymes are capable of modulating the host immune response. They can cleave immune factors involved in immune recognition and activation, such as immunoglobulins, complement proteins, and cytokines. This modulation attenuates immune responses, promoting fungal survival in the host (Almeida *et al*., 2015).

### 1.3.4 Growth at Host Body Temperature

*Cryptococcus neoformans* has a remarkable ability to grow and multiply at physiological host temperatures, typically 37°C. This heat tolerance is important for the successful establishment and progression of cryptococcal infections. Adaptation to host body temperature involves complex molecular and cellular mechanisms that enable fungi to overcome heat stress and exploit the host environment. The ability to grow at host body temperature allows

*Cryptococcus neoformans* to colonize various niches of the host including the respiratory tract, central nervous system, and other organs. The fungus can survive and proliferate in the host's tissues, causing the infection to spread. The immune evasion of *Cryptococcus neoformans* is facilitated at 37°C. Thermal adaptation allows fungi to multiply in host phagocytic cells, such as macrophages, altering their antimicrobial functions. Intracellular growth also protects the fungus from host immune effectors, facilitating its survival and dissemination (McCusker *et al.,* 1994). Growth at 37°C induces expression of different virulence factors in *Cryptococcus neoformans*. The thermal shift triggers the upregulation of genes involved in capsule formation, melanin production, stress response, and other pathogenic traits. The increased expression of these virulence genes contributes to the pathogenicity of the fungus (Fan *et al*., 2005). Growth at host body temperature promotes interactions between *Cryptococcus neoformans* and host cells. The fungus is able to modulate host cell signaling pathways, induce inflammatory responses, and manipulate host immune responses through the expression of temperature-sensitive factors. . These interactions facilitate the formation and progression of cryptococcal infections (O'Meara and Alspaugh, 2012). *Cryptococcus neoformans* uses a variety of molecular mechanisms to adapt to host body temperature, including:

- Differential gene expression: The Fungi undergo extensive transcriptional reprogramming at elevated temperatures, leading to the expression of heat shock proteins, chaperones, and other factors involved in heat tolerance.

- Membrane remodeling: *Cryptococcus neoformans* modifies its membrane composition and lipid metabolism to maintain membrane integrity and functionality under thermal stress.

- Signal transduction pathways: The cAMP-PKA pathway, the high-osmolarity glycerol (HOG) pathway and other signaling cascades play important roles in the regulation of heat tolerance in *C. neoformans.*

## 1.4 ENVIRONMENTAL INFLUENCE ON MATING TYPES IN *CRYPTOCOCCUS NEOFORMANS*

*Cryptococcus neoformans*, a fungal pathogen, exhibits a unique mating system influenced by many environmental factors (Lin and Heitman, 2006). Mating partner availability, nutrient availability, temperature, and specific environmental cues play an important role in shaping the mating behavior and population structure of this pathogenic fungus (Nielsen and Heitman, 2007).

The availability of opposite mating types is important for sexual reproduction in *Cryptococcus neoformans* (Kwon-Chung and Bennett, 1978). When MATα and MATa cells are in close proximity, they can engage in sexual reproduction, leading to the generation of genetically diverse offspring (Nielsen *et al*., 2003). The presence of both mating types in the environment is influenced by factors such as population density, dispersal patterns, and host interactions.

In environment with a high concentration of *Cryptococcus neoformans* cells, the likelihood of encountering cells of the opposite mating type increases, promoting sexual reproduction (Lin and Heitman, 2006). Bird nesting sites, such as tree hollows and soil contaminated with bird droppings, are important reservoirs of *Cryptococcus neoformans*, creating a favorable environment for the presence of MATα and MATa cells (Lin and Heitman, 2006). Bird-related activities, such as perching and nesting, can contribute to fungal dispersal and increase the likelihood of encountering opposing mating patterns.

### 1.4.1 Nutrient induced stress

Nutrient induced stress plays an important role in determining the mating behavior of *Cryptococcus neoformans* (Alby and Bennett, 2011). Nutrient limitation can induce a transition from bipolar mating (mating between MATα and MATa cells) to unisexual reproduction (mating between cells of the same mating type) (Alby and Bennett). , 2011). Unisexual reproduction allows *Cryptococcus neoformans* to reproduce even in the absence of opposite mating types, increasing their chances of survival and adaptation in resource-constrained environments.

Under nutrient-rich conditions, *Cryptococcus neoformans* tends to promote bipolar mating (Alby and Bennett, 2011). This preference may be due to the benefit of genetic recombination and the increased genetic diversity resulting from mating between opposite mating types (Nielsen *et al*., 2003). However, when faced with nutrient scarcity, the fungus can switch to unisexual reproduction as a means to maximize its reproductive potential (Alby and Bennett, 2011).

### 1.4.2 Temperature

Temperature is another environmental factor affecting the mating behavior of *Cryptococcus neoformans* (Wang *et al*., 2012). Different temperature ranges can affect the efficiency and frequency of mating events, thereby affecting the distribution and prevalence of mating types in the population.

Studies have shown that the optimal temperature for sexual reproduction in *Cryptococcus neoformans* usually ranges between 22°C and 30°C, depending on strain and environmental conditions (Wang *et al*., 2012). At temperatures below or above this range, mating efficiency can be reduced, leading to a potential bias in the distribution of mating types in the population.

### 1.4.3 Signaling Molecules and Environmental Cues

*Cryptococcus neoformans* can sense and respond to specific environmental cues and signaling molecules, which may influence its mating behavior (Hsueh *et al*., 2006). These cues include pheromones, nutritional signals, and factors secreted by other microorganisms.

Pheromones, produced by MATα and MATa cells, play an important role in the recognition and attraction between opposite mating types (Hsueh *et al*., 2006). Sensing pheromones by cell surface receptors initiates a signaling cascade that activates the mating response (Hsueh *et al*., 2006). The production and sensing of pheromones allows *Cryptococcus neoformans* to efficiently coordinate mating events. In addition to pheromones, nutritional signals also play a role in regulating mating types (Alby and Bennett, 2011). Specific nutritional conditions can induce changes in gene expression and signal transduction, ultimately influencing the choice between bipolar mating and unisexual reproduction (Alby and Bennett, 2011). .

In addition, interactions with other microorganisms in the environment can also influence the mating behavior of *Cryptococcus neoformans* (Lin and Heitman, 2006). For example, the presence of certain other bacteria or fungi can induce signaling molecules that influence the mating behavior of *Cryptococcus neoformans*, potentially altering the distribution and prevalence of mating types distribution in the population (Lin and Heitmann, 2006).

## 1.5 GENETIC INFLUENCE ON MATING PATTERN IN *CRYPTOCOCCUS NEOFORMANS*

The determination and inheritance of mating types in *Cryptococcus neoformans* is largely influenced by genetic factors. The presence of two distinct mating types, MATα and MATa, and the regulatory genes associated with their expression play important roles in the mating behavior and population structure of this fungal pathogen (Kwon -Chung and Bennett, 1978).

### 1.5.1 Genetic diversity and evolution

Genetic diversity within the MAT locus and surrounding regions contributes to the evolutionary dynamics of mating types in *Cryptococcus neoformans* (Hagen and Kwon-Chung, 2010). Recombinant events and genetic exchange between the MATα and MATa alleles lead to the generation of new genotypes and maintenance of genetic diversity in the population.

Studies have revealed the presence of recombination hotspots adjacent to the MAT locus, indicating a potential for genetic exchange between different mating patterns (Hsueh *et al*., 2006). These recombination events contribute to the generation of new combinations of mating type-specific genes and the production of offspring with diverse genetic profiles.

In addition, the genetic makeup of the MAT locus and the mating type switching machinery can evolve in response to selective pressures, including host immune responses and antifungal treatments. Mutations in genes involved in mating type determination and switching can influence the mating behavior of *Cryptococcus neoformans* and affect its survival and pathogenicity (Nielsen *et al*., 2003).

## 1.6 SUSCEPTIBILITY AND RESISTANCE OF CRYPTOCOCCUS NEOFORMANS TO FLUCONAZOLE

*Cryptococcus neoformans*, a fungal pathogen that causes cryptococcal disease, may show varying degrees of susceptibility or resistance to fluconazole, an antifungal commonly used to treat cryptococcal infections. The susceptibility profile of *Cryptococcus neoformans* to fluconazole is influenced by several factors, including the genetic makeup of the strain, the presence of specific resistance mechanisms, and the organism's history of drug exposure.

### 1.6.1 Genetic Factors

Genetic factors an important role in determining the susceptibility or resistance of *Cryptococcus neoformans* play to fluconazole. Gene mutations associated with drug target sites or drug efflux pumps can confer resistance to antifungal drugs. For example, alterations in the target enzyme Erg11 (lanosterol 14α-demethylase) can reduce fluconazole's affinity for the enzyme, resulting in reduced susceptibility (Perfect *et al.,* 2010).

In addition, overexpression of efflux pumps, such as ATP-binding cassette (ABC) transporters encoded by genes such as CnAFR1 and CnCDR1, can result in the active extrusion of fluconazole from the fungal cell, reducing intracellular drug accumulation and contributing to drug resistance (Sionov *et al.,* 2009).

### 1.6.2 Mechanisms of Resistance:

*Cryptococcus neoformans* may use different mechanisms to develop resistance to fluconazole. Besides alteration of the target site and overexpression of the efflux pump, other mechanisms such as alterations in membrane composition, upregulation of stress response pathways, and changes in expression ergosterol biosynthetic gene have been associated with fluconazole resistance (Chang *et al.,* 2018). The accumulation of genetic alterations, including point mutations, insertions or deletions, in these pathways can lead to altered drug susceptibility and reduced therapeutic efficacy of fluconazole against *Cryptococcus neoformans*.

## 1.7 ANTIFUNGAL SUSCEPTIBILITY TESTING

To determine the susceptibility of *Cryptococcus neoformans* isolates to fluconazole, antifungal susceptibility testing methods, such as the broth microdilution method recommended by the Clinical and Laboratory Standards Institute on Broth microdilution are employed. These tests

provide quantitative measurements of minimum inhibitory concentrations (MICs), which help guide therapeutic decisions and detect emerging drug resistance.

The susceptibility or resistance of *Cryptococcus neoformans* to fluconazole is influenced by a combination of genetic determinants, including mutations in drug target sites and efflux pump genes, as well as other mechanisms such as changes in membrane composition and stress response pathways. Clinical isolates can exhibit varying levels of susceptibility or resistance, and combination therapy approaches are employed to overcome resistance mechanisms. Antifungal susceptibility testing plays an important role in guiding treatment decisions. Understanding the factors contributing to fluconazole susceptibility or resistance in *Cryptococcus neoformans* is essential for effective management and control of cryptococcal infections.

## 1.8 STATEMENT OF THE PROBLEM

*Cryptococcus neoformans* is a pathogenic fungus that can cause severe infections, especially in immunocompromised individuals such as those with HIV/AIDS (Perfect *et al*., 2010). The mating types of *C. neoformans*, designated as MAT α and MAT a, play a crucial role in the fungus's sexual reproduction and overall pathogenicity (Kwon-Chung *et al.,* 2017). Understanding the genetic and environmental factors that influence the expression of mating types in *C. neoformans* is essential for elucidating its transmission dynamics and the development of targeted antifungal therapies.

While previous studies have identified the genetic basis of mating type determination in *C. neoformans*, the exact mechanisms underlying the switch between α and a mating types remain poorly understood (Xu *et al.,* 2007). Additionally, the influence of environmental factors on the regulation of mating type expression in *C. neoformans* has not been extensively explored.

Investigating these aspects is crucial for gaining insights into the adaptive potential and virulence of this pathogen.

Therefore, the problem addressed in this project is the need to isolate DNA from *C. neoformans* samples and analyze the mating types of the isolates, while considering the genetic and environmental influences on mating type expression. By addressing this problem, we aim to enhance our understanding of *C. neoformans* pathogenesis and the factors that contribute to its infectivity.

## 1.9 JUSTIFICATION OF THE RESEARCH

The research on isolating DNA and analyzing mating types in *Cryptococcus neoformans* is important due to its clinical significance in improving patient outcomes for cryptococcosis. It also provides insights into the transmission dynamics, adaptation, and virulence of *C. neoformans*, which can aid in controlling the spread of the pathogen. The research has implications for public health by informing surveillance and outbreak investigations. Furthermore, the knowledge gap in understanding the genetic and environmental influences on mating types motivates the need for this research, which can contribute to the broader field of fungal biology and pathogenesis.

## 1.10 AIM OF THE STUDY

The aim is this study is the isolation of DNA and investigation of the genetic and environmental influences on the mating types of *Cryptococcus neoformans*.

## 1.11 OBJECTIVES OF THE STUDY

The objectives of this study are:

i. Extraction of DNA from *Cryptococcus neoformans*

ii. Differentiation of the mating types (MATα and MATa) of the isolated *C. neoformans* strains through reaction to fluconazole and polymerase chain reaction (PCR); and investigate the environmental influence using medium containing hydrogen peroxide and sodium nitrate as a means oxidative and nitrosative stress in order to further differentiate the mating types.

# CHAPTER TWO

## 2.0 MATERIALS AND METHODS

### 2.1 MATERIALS USED

Fifteen environmental samples were collected from three different markets; Egor market, Uselu market and New Benin. Clinical isolates used were already isolated from previous work (Eboigbe and Usiosefe, 2019) from the University of Benin Teaching Hospital (UBTH) and so did not require medical ethics to acquire them.

### 2.2 METHODS

#### 2.2.1 PREPATION OF MEDIA USED

##### 2.2.1.1 Yeast Extract Peptone Dextrose Broth:

This medium was prepared by weighing 5 grams of glucose, 5 grams of peptone, and 2.5 grams of yeast extract and dissolving in 250 millilitre of distilled water. The solution was properly mixed and poured into a conical flask. It was then sterilized for 30minutes at 15psi in an autoclave.

#### 2.2.1.2 Yeast Extract Peptone Dextrose Agar:

This medium was prepared by weighing 5 grams of glucose, 5 grams of peptone, 2.5 grams of yeast extract, 5 grams of agar and dissolving in 250 millilitre of distilled water. The solution was properly mixed and poured into a conical flask. It was then sterilized for 30minutes at 15psi in an autoclave.

### 2.2.3 Urease

This medium was prepared by weighing 12 grams of urea agar base medium in 500ml of water. The solution was sterilized in an autoclave at 15psi for 30minutes. The solution was cooled to 40°C and 12ml of sterile 40% urea solution prepared by dissolving urea powder weighing 5g in 12ml of water was added. Mixture was properly mixed and dispensed into McCartney bottles and set in a slanted position.

## 2.3 SAMPLING AND ISOLATION

### 2.3.1 Environmental Sampling

The environmental samples used in this work were collected from Pigeon droppings at New Benin market. The Pigeon droppings were collected aseptically using absolute ethanol, spatula and cotton wool in sterilized McCartney bottles.

Figure 2.1 shows the geographical map of areas of sampling collection in Benin City, Edo state.

The areas where the sampling was carried out include New Benin market, Uselu market and Egor market.

Figure 3.1: Map of Edo state showing market areas where environmental sampling was carried out in Benin City.

### 2.3.2 Isolation of *Cryptococcus neoformans* from samples

The environmental isolates were obtained by collecting approximately 1 gram sample of pigeon droppings from each McCartney bottles. Yeast extract dextrose broth was poured into sterilized McCartney bottles and they were labeled. Under sterile conditions, the samples were inoculated into the broth medium. The inoculated samples were incubated at 37°C for 24 hours in a Gallenhamp incubator allowing the growth of *Cryptococcus neoformans* if present. Afterwards, Yeast extract dextrose agar was poured into Petri dishes. The plates were allowed to solidify after which they were inoculated with 0.5ml broth cultures. The plates were incubated at 37°C for 48 hours. Observations were made for the growth of *C. neoformans* (Oghomwenakhin and Eboigbe, 2020).

In order to obtain pure colonies, isolates which showed positive growth similar to yeast like colonies of *C. neoformans* were selected and sub-cultured in fresh yeast extract broth media. In order to accomplish this, under sterile condition, a single colony was selected from the agar plates and it was inoculated into broth media using a laminar flow chamber and a flame lamp. These broth cultures as appropriately labeled were transferred to the incubator at 37°C for 24 hours. After the incubation period, a sterile pipette was used to inoculate 0.25 ml of inoculum into agar plates. These were incubated at 37°C for another 48 hours. Colonies which were positive for the growth of *C. neoformans* were selected. (Oghomwenakhin and Eboigbe, 2020).

## 2.4 CONFIRMATION OF *C. NEOFORMANS* ISOLATES

### 2.4.1 Urease Test

This medium was used for the confirmation of *C. neoformans* isolates. The medium was poured into McCartney bottles and labeled. Eppendorf containing sample were introduced into McCartney bottles and set in a slanted position for 24 hours. Medium which turned pink were confirmed to have *C. neoformans*. Urease medium containing *C. neoformans* were transferred to McCartney bottles containing yeast extract dextrose broth using sterilized tooth picks. The bottles were then kept in an incubator (Oghomwenakhim and Eboigbe, 2020).

### 2.4.2 Niger Seed Agar

Niger seed agar was used for the confirmation of *C. neoformans* isolates. The medium was poured in Petri dishes and labeled. The *C. neoformans* isolates on yeast extract peptone dextrose agar were sub-cultured first into yeast extract broth and incubated at 37°C for 24 hours. The broth cultures were then inoculated on niger seed agar plates and incubated at 37°C for at least 48 hours. Isolates which grew and produced brownish melanin were confirmed as *C. neoformans* isolates. The confirmed isolates were then maintained on fresh yeast extract broth with sub culturing every three weeks (Staib, 2005; Stranchan, Yu and Blank, 1971).

## 2.5 ANTIFUNGAL SUSCEPTIBILITY TESTING

Using fluconazole as the antifungal agent, antifungal susceptibility tests were carried out using two of the confirmed isolates. This tests were carried out according to the M27-A2 document from the Clinical and Laboratory Standards Institute on Broth microdilution method. The antifungal concentration used was 16 µl/ml. The environmental isolate NBM5 and the clinical isolate FBC were used for the antifungal susceptibility testing. Antifungal susceptibility testing was carried out by obtaining the antifungal agent fluconazole. The concentration of fluconazole used was 50mg. For this test, the concentration of fluconazole prepared was 16 µg/mlk. 50mg of

fluconazole was dissolved in 0.002 ml of sterile water and mixed thoroughly. Using the sterile dilution principle, the environmental isolate NBM5 and the clinical isolate FBC were subcultured into yeast extract peptone dextrose media and they were incubated at $37^0$C for 24 hours. The inoculum volume used for sub-culturing was 50 µl. Each eppendorf tube used for dilution contained 1 µl of yeast extract peptone dextrose broth medium. Yeast extract peptone dextrose agar medium was prepared and plates of volume 9ml were poured with the required volume for the concentration of fluconazole. The agar plates was then inoculated with 100 µl of the inoculum concentration (NBM5 and FBC isolates) according to Eboigbe and Usiosefe (2019). They were incubated for 24 hours at $37^0$C. After 24 hours, the number of colonies was recorded. Positive control (+C) was prepared with yeast extract peptone dextrose agar lacking the fluconazole drug. Two agar plates were inoculated with inocula of the two isolates and incubated at $37^0$C for 24 hours. The negative control (-C) was prepared by pouring yeast extract peptone dextrose agar plate with fluconazole but not inoculated. The negative control was incubated at $37^0$C for 24 hours. Colonies were expected from the positive control while no colony was expected from the negative control (Eboigbe and Usiosefe, 2019).

## 2.6 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MICS)

Determination of the MICs was according to (Eboigbe and Usiosefe, 2019). The concentration that inhibited more than 50% of growth when compared visually with the positive control was taken as the MIC value.

## 2.7 DETERMINATION OF MATING TYPES

Mating types of both isolates were determined according to methods outlined by Eboigbe and Usiosefe, (2019). Differences in their susceptibilities to fluconazole were used to determine mating type.

## 2.8 ENVIRONMENTAL INFLUENCE ON THE MATING TYPE IN CRYPTOCOCCUS NEOFORMANS

Two strains of *Cryptococcus neoformans* var. *neoformans* (serotype D) were analysed according to Samarasinghe *et al.* (2018). The environmental isolate NBM5 and the clinical isolate FBC were used for this analysis. Oxidative and nitrosative stress was imposed on these isolates to check for how the environment influences the mating type of both clinical isolate and the environmental isolate. Oxidative and nitrosative stress was induced by using hydrogen peroxide ($H_2O_2$) and sodium nitrite ($NaNO_2$) respectively. To create oxidative stress, hydrogen peroxide ($H_2O_2$) was added to yeast extract peptone dextrose agar in one of the three concentrations (50 µl, 100 µl and 150 µl). To create nitrosative stress, sodium nitrite salt ($NaNO_2$) was added to yeast extract peptone dextrose agar in one of the three concentrations (50 µl, 100 µl and 150 µl). Using the sterile dilution principle, the environmental isolate NBM5 and the clinical isolate FBC were subcultured into yeast extract peptone dextrose media and they were incubated at $37^0$C for 24 hours. The inoculum volume used for sub-culturing was 50 µl. Each eppendorf tube used for dilution contained 1 µl of yeast extract peptone dextrose broth medium. Yeast extract peptone dextrose agar medium was prepared and plates of volume 9ml were poured with the required volume for the concentration of hydrogen peroxide ($H_2O_2$) and sodium nitrite ($NaNO_2$). The agar plates was then inoculated with 100 µl of the inoculum concentration (NBM5 and FBC isolates). They were incubated for 72 hours at $37^0$C. After 72 hours, the number of colonies was recorded.

## 2.9 EXTRACTION OF DNA

The isolate was sub-cultured overnight in preparation for DNA extraction. 1 µl (microlitre) of the sub-cultured isolate was transferred into a sterilized eppendorf using micropipette. The eppendorf was centrifuged at 3,000 rpm (revolutions per minutes) for 1 minutes to pellet the cells and the supernatant was carefully removed without disturbing the cell pellet. This step was repeated three times after which 500 µl (microlitre) of the extraction buffer was the added to the eppendorf containing the extraction buffer. 350 µl of buffered phenol was then added and homogenously mixed by inverting the eppendorf 3 – 6 times. Following this, 150 µl of chloroform:isoamyl alcohol (24 : 1) was added and mixed by inverting the eppendorf 3 – 6 times. Centrifuge at 13000 g for 1 hour. After centrifuging, the upper aqueous phase was carefully removed immediately and transferred to a new eppendorf tube. 25 µl RNAase solution (stock 10 mg/ml) was added to the eppendorf (the stock solution became turbid) and it was incubated at 37°C for 1 hour. After incubating, 350 µl of buffered phenol was again added and homogenously mixed by inverting the eppendorf 3 – 6 times. 150 µl of chloroform:isoamyl alcohol (24 : 1) was then added and mixed by inverting the eppendorf 3 – 6 times. The upper phase was then carefully transferred to a new eppendorf and mixed with 40% volume 3M Na-Aceteate and 60% volume of isopropanol. This was then incubated at room temperature for 1 hour to precipitate the DNA. Following this, the eppendorf was centrifuged at 13000 g for 5 minutes and the supernatant carefully decanted without disturbing the DNA pellet. The DNA pellet was then rinsed by adding 70% ethanol after which it was centrifuged for 10 minutes. The ethanol was discarded and any residual ethanol by inverting the eppendorf. The DNA pellet was allowed to dry at room temperature (Bolano *et al*., 2001).

Under specific conditions, run gel electrophoresis in order to check the size and quality of the DNA.

## 2.10 STATISTICAL ANALYSIS

Statistical analysis was carried out using t-Test: Two-Sample Assuming Equal Varience at a significance level of $\alpha = 0.05$

# CHAPTER THREE

## 3.0 RESULTS

Figure 3.1 shows the geographical map of areas of sampling collection in Benin city, Edo state. The areas where the sampling was carried out include New Benin market, Uselu market and Egor market.

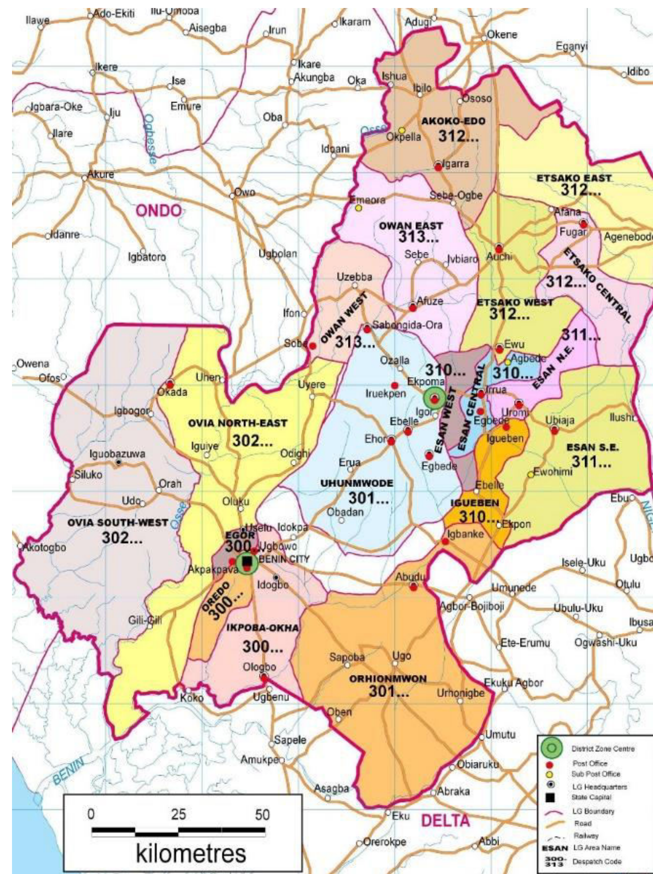



Figure 3.1: Map of Edo state showing market areas where environmental sampling was carried out in Benin city.

Fifteen environmental samples were collected and tested for the presence of *C. neoformans.* Two clinical samples were also tested for *C. neoformans*. The isolates were first confirmed using urease and then on Niger seed agar with growth at 37°C and production of a brownish colouration.

Environmental isolates were; Eg1, Eg2, Eg3, Eg4, Eg5, Uselu1, Uselu2, Uselu3, Uselu4, Uselu5, NBM1, NBM2, NBM3, NBM4, NBM5 as shown in Table 3.1. Clinical isolates used were already isolated by previous work (Eboigbe and Usiosefe, 2019) from the University of Benin Teaching Hospital (UBTH) and so did not require medical ethics to acquire them. They are 4832, FBC as seen in Table 3.2.

Table 3.1 shows the response of the environmental isolates to urease test. Isolates which turned pinkish colouration were positive (+).

**Table 3.1:** Environmental samples and isolates that indicated the presence (+) or absence(-) of *C. neoformans* isolates

| Site of Collection | Sample/isolates | Presence of *C. neoformans* |
|---|---|---|
| Egor market | Eg1 | + |
| Egor market | Eg2 | + |
| Egor market | Eg3 | + |
| Egor market | Eg4 | + |
| Egor market | Eg5 | + |
| Uselu market | Uselu1 | + |
| Uselu market | Uselu2 | + |
| Uselu market | Uselu3 | + |
| Uselu market | Uselu4 | _ |
| Uselu market | Uselu5 | _ |
| New Benin market | NBM1 | + |
| New Benin market | NBM2 | + |
| New Benin market | NBM3 | + |
| New Benin market | NBM4 | _ |
| New Benin market | NBM5 | + |

Note: + represents isolates that were positive for *C. neoformans*

- represents isolates that were negative isolates

Table 3.2 shows the response of the clinical isolates to urease test. Isolates which turned pinkish colouration were positive (+).

**Table 3.2:** Clinical samples and isolates that indicated the presence(+) or absence(-) of *C. neoformans* isolates.

| Site of Collection | Sample/isolates | Presence of *C. neoformans* |
|---|---|---|
| UBTH | 4832 | + |
| UBTH | FBC | + |

Note: + represents isolates that were positive for *C. neoformans*

- represents isolates that were negative isolates

The image in plate 3.1 shows the appearance of *Cryptococcus neoformans* in Yeast extract peptone dextrose agar. It is cream in colour.

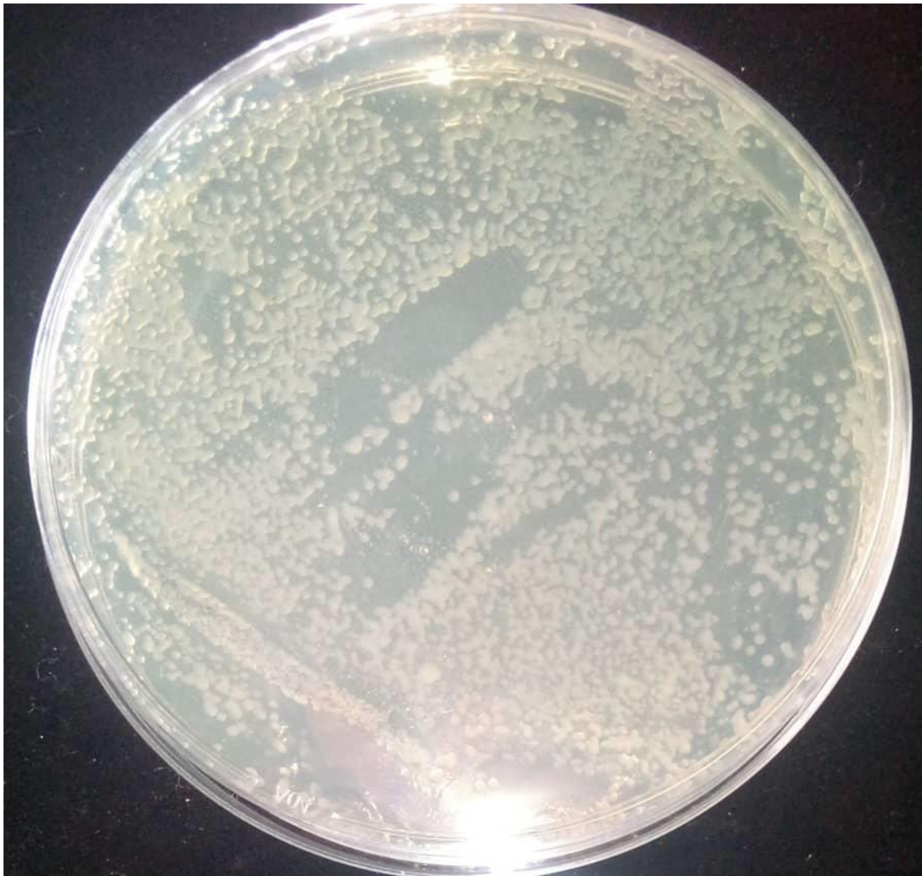


**Plate 3.1: Yeast extract peptone dextrose agar containing *Cryptococcus neoformans***

To confirm the presence of *Cryptococcus neoformans* in the isolate above, they were subjected to urease test. Positive results showed pinkish colouration.

**Plate 3.3: Urease medium showing pinkish colouration confirming the presence of *Cryptococcus neoformans***

From the above, isolates which were positive to urease test were cultured on Niger seed agar. The production of a brownish colouration confirmed the presence of *Cryptococcus neoformans.*

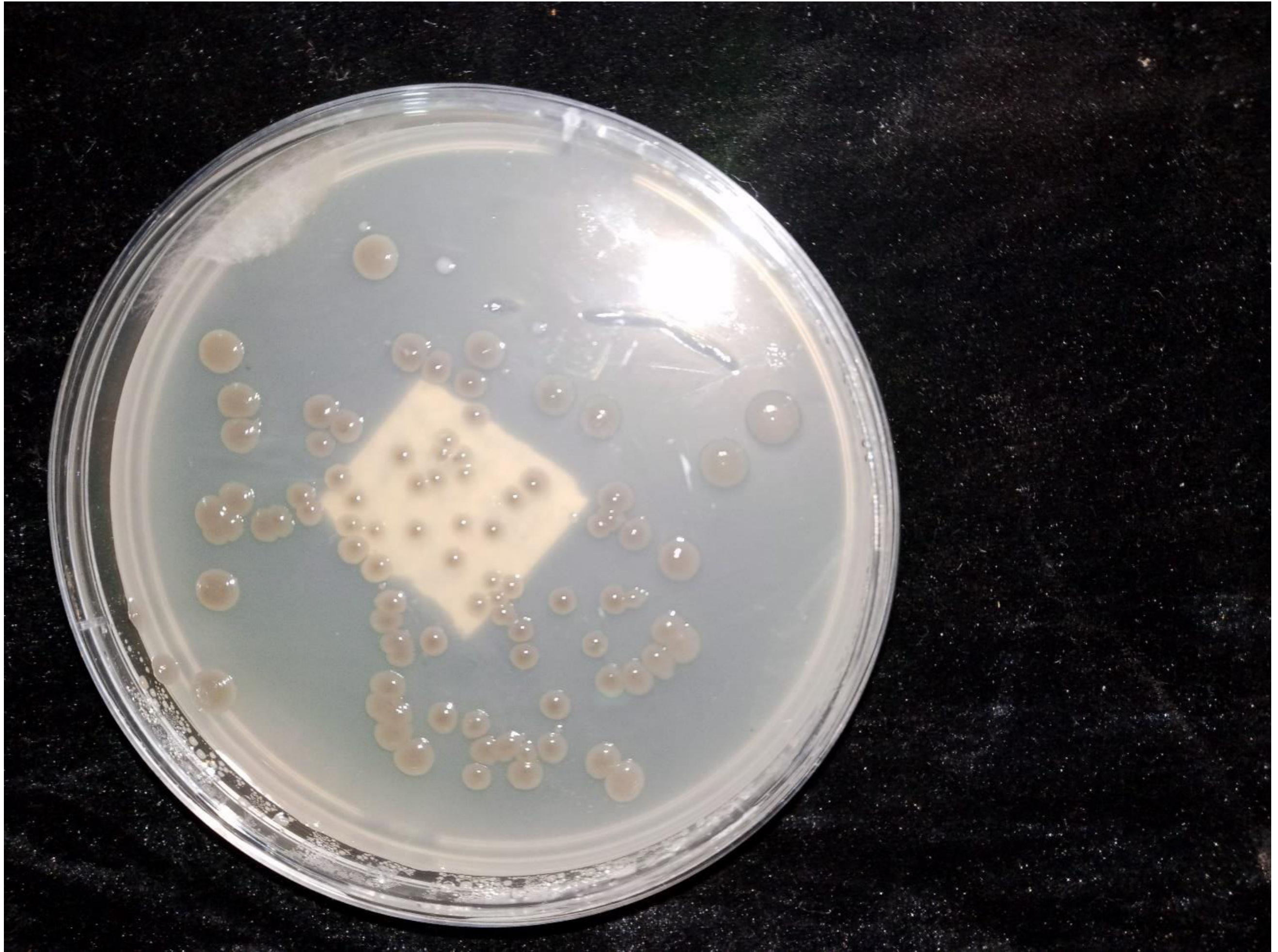
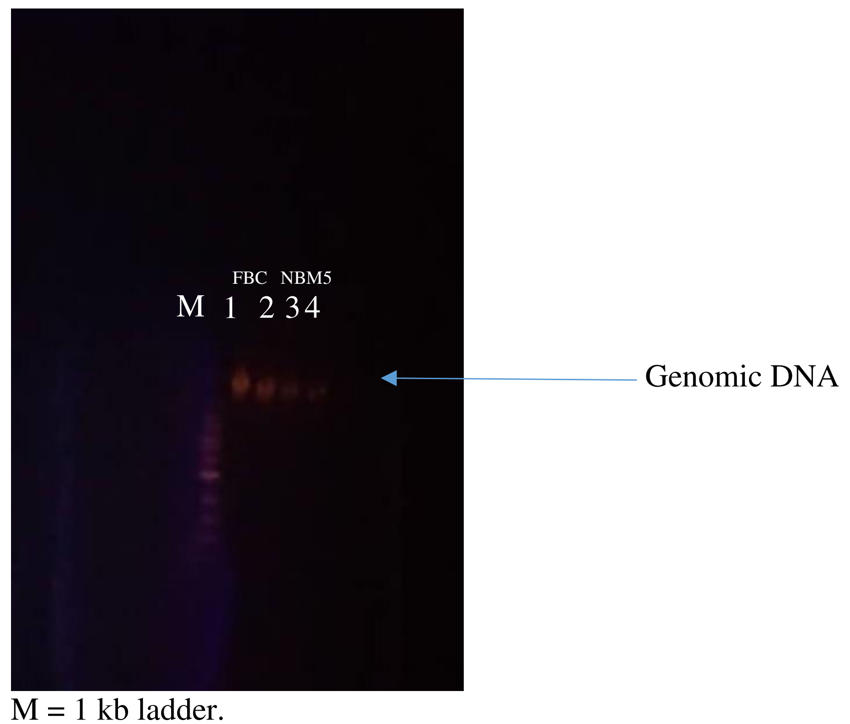


**Plate 3.4: Niger seed agar containing *Cryptococcus neoformans***

Plate 3.5 shows the DNA isolated from four isolates; two clinical isolates (FBC1 and FBC2) and two environmental isolates (NBM5-1 and NBM5-2). The different sizes of DNA ranges from 20 kb to about 250 kb. This genomic DNA shows a size far higher than 20kb and therefore indicates that the total genomic DNA of *Cryptococcus neoformans* is higher than 20 kb.

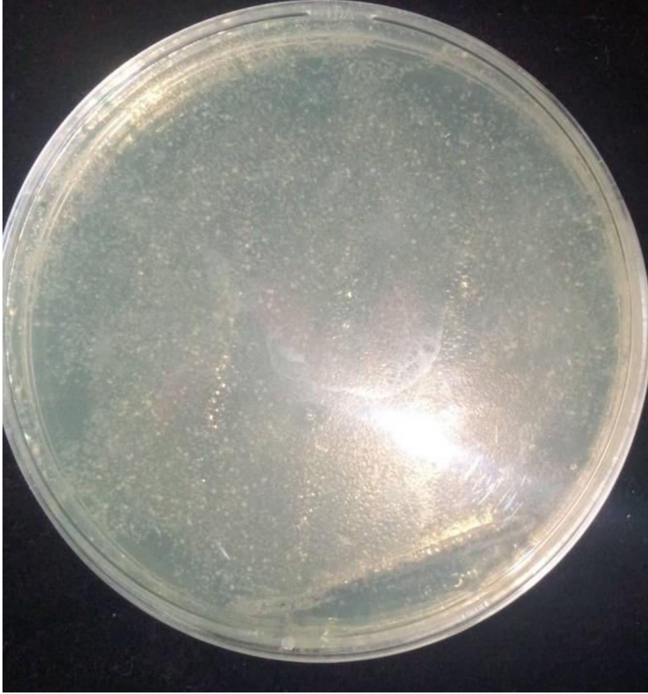


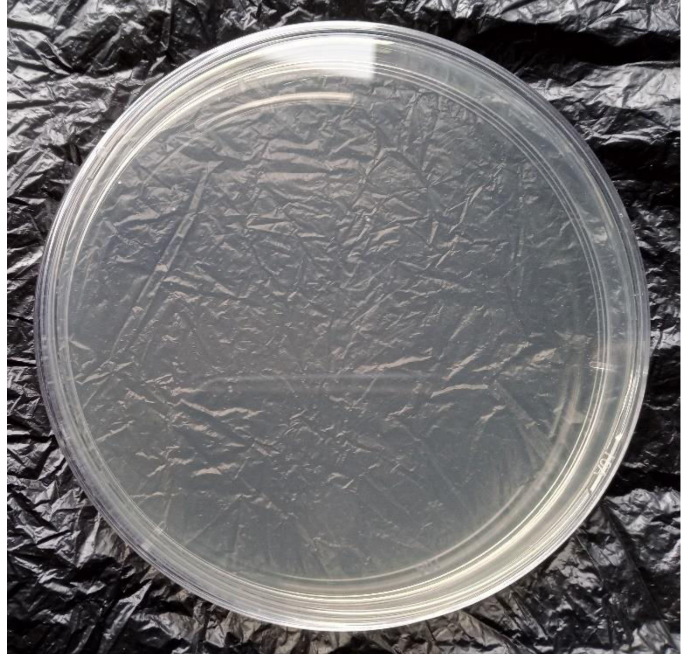

M = 1 kb ladder.

**Plate 3.5: Total genomic DNA of *Cryptococcus neoformans***

## ANTIFUNGAL SUSCEPTIBILITY PROFILE

Results obtained from fluconazole antifungal susceptibility testing are outlined in table 3.3 and 3.4. The result shows that from the antifungal susceptibility test and the statistical analysis outlined in table 3.5, the two strains, FBC (Clinical isolate) and NBM5 (Environmental isolate) acts as if they are of the same mating type. Based on previous work done (Eboigbe and Usiosefe, 2019), it shows that both Clinical isolate FBC and Environmental Isolate NBM5 are of the same mating type.





**a** **b**

**Plate 3.6: Control for antifungal susceptibility testing**

a: Positive control (+C)

b: Negative control (-C)

Table 3.3 shows the response of the clinical isolate FBC to antifungal susceptibility test using Fluconazole as the antibiotic agent.

**Table 3.3: Response of Clinical isolate FBC to Fluconazole: Colony count per strain**

| Concentration of Fluconazole | | | Inoculum Concentration | | |
|---|---|---|---|---|---|
| (μg/ml) | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ |
| 16 | 619 | 336 | 233 | 45 | 6 |

Table 4 shows the response of the environmental isolate NBM5 to antifungal susceptibility test using Fluconazole as the antibiotic agent.

**Table 3.4:** Response of Environmental isolate NBM5 to Fluconazole: Colony count per strain

| Concentration of Fluconazole | Inoculum Concentration | | | | |
|---|---|---|---|---|---|
| (μg/ml) | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ |
| 16 | 422 | 128 | 33 | 31 | 10 |

**Table 3.5:** t-Test: Two-Sample Assuming Equal Variances

| | *FBC* | *NBM5* |
|---|---|---|
| Mean | 247.8 | 124.8 |
| Variance | 61345.7 | 29685.7 |
| Observations | 5 | 5 |
| Pooled Variance | 45515.7 | |
| Hypothesized Mean Difference | 0 | |
| Df | 8 | |
| t Stat | 0.911579 | |
| P(T<=t) one-tail | 0.194316 | |
| t Critical one-tail | 1.859548 | |
| P(T<=t) two-tail | 0.388633 | |
| t Critical two-tail | 2.306004 | |

At a significance level of α = 0.05, we failed to reject the null hypothesis. Therefore, based on the available data, there is not enough evidence to conclude that there is a significant difference in virulence between the clinical isolate FBC and the environmental isolate NBM5.

Table 6 shows the response of the environmental isolate NBM5 to environmental stressors using Sodium Nitrite ($NaNO_2$) as the oxidizing agent.

**Table 3.6:** Response of Environmental isolate NBM5 to Sodium Nitrite ($NaNO_2$): Colony count per strain

| Concentration of hydrogen peroxide (30%) ($H_2O_2$) (μl) | Number of colonies |
|---|---|
| 50 | 281 |
| 100 | 232 |
| 150 | 261 |

Table 3.7 shows the response of the environmental isolate NBM5 to environmental stressors using hydrogen peroxide ($H_2O_2$) as the nitrosative agent

**Table 3.7:** Response of Environmental isolate NBM5 to hydrogen peroxide ($H_2O_2$): Colony count per strain

| Concentration of hydrogen peroxide (30%) ($H_2O_2$) (μl) | Number of colonies |
|---|---|
| 50 | 0 |
| 100 | 0 |
| 150 | 0 |

Table 3.8 shows the response of the Clinical isolate FBC to environmental stressors using Sodium Nitrite ($NaNO_2$) the nitrosative agent.

**Table 3.8:** Response of Clinical isolate FBC to Sodium Nitrite ($NaNO_2$): Colony count per strain

| Concentration of hydrogen peroxide (30%) ($H_2O_2$) (μl) | Number of colonies |
|---|---|
| 50 | 312 |
| 100 | 0 |
| 150 | 373 |

Table 3.9 shows the response of the Clinical isolate FBC to environmental stressors using Hydrogen Peroxide ($H_2O_2$) as the oxidizing agent.

**Table 3.9:** Response of Clinical isolate FBC to Hydrogen Peroxide ($H_2O_2$): Colony count per strain

| Concentration of hydrogen peroxide (30%) ($H_2O_2$) (μl) | Number of colonies |
|---|---|
| 50 | 0 |
| 100 | 0 |
| 150 | 0 |

Figure 3.2 shows that from the beginning to the end, there is actually no real variation between the Clinical isolate FBC and the Environmental Isolate NBM5 in their reaction to substance being used on them. Their physical reaction may vary at one point or the other but their overall result shows that there is no variability among them in as much as they are of the same type. This is why they intercept at the beginning and towards the end.

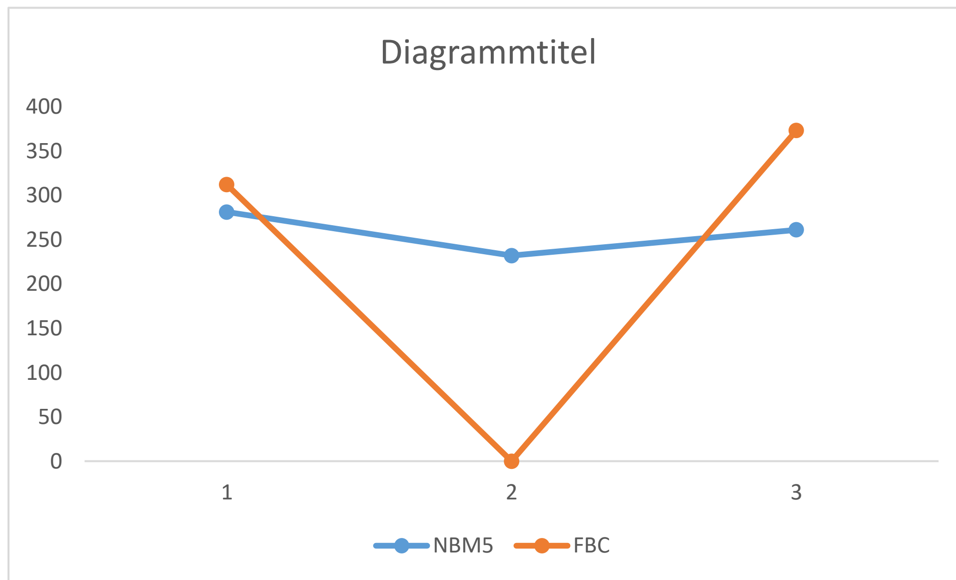



**Figure 3.2: Graphical representation of the response of the Clinical isolate FBC and Environmental isolate NBM5 to nitrosative stress.**

# CHAPTER FOUR

## 4.0 DISCUSSION

*Cryptococcus neoformans* isolates were identified by the appearance of their growth on Yeast extract peptone dextrose agar. The yeast like growth of the cream coloured colonies aligned with the work done by Liaw and Hsueh. (2009). Isolates that were able to grow at a temperature of $37^{o}C$ after incubation for a period of 48 hours were selected for confirmation on Niger seed agar which is used as selective medium for *Cryptococcus neoformans*. The growth of this isolates was confirmed based on the formation of melanin (brown pigment). The formation of brown colonies on niger seed agar was used in the identification of *Cryptococcus neoformans* on the basis of thermotolerance at a temperature of $37^{o}C$ (Lazéra *et al.,* 1997).

The clinical and environmental isolates were selected for antifungal susceptibility testing due to their performance during isolation and confirmation. The susceptibility of both isolates to fluconazole, revealed that both the clinical isolate (FBC) and environmental isolate (NBM5) showed similar response suggesting that both isolates may be of the same mating type. Further investigation carried out by subjecting both isolates to environmental stressors also revealed similar result as above. The environmental stressors used were sodium nitrate ($NaNO_2$) inducing nitosative stress and hydrogen peroxide ($H_2O_2$) inducing oxidative stress.

Studies have shown that the distribution of mating types could vary across geographic regions, leading to variations in genetic diversity of *Cryptococcus neoformans* populations. These genetic

diversity have implications in virulence, antifungal susceptibility, and clinical outcomes (Litvintseva *et al*., 2006; Chen *et al*., 2015). Environmental factors play an important role in shaping the distribution of mating types in *Cryptococcus neoformans*. However, in this research, the various genetic (susceptibility to Fluconazole) and environmental factor could not differentiate the isolates into different mating types leading to the conclusion that the two major isolates used are of the same mating type. To further proof the authenticity of both isolates having the same mating type, DNA was isolated from the clinical and environmental isolates for molecular analysis using polymerase chain reaction (PCR) in the near future. Specific environmental conditions, such as bird droppings or decaying plant matter, have been observed to favor the proliferation of certain mating types. Mating types in *Cryptococcus neoformans* are associated with differences in clinical features, including disease presentation, severity, and outcome. Studies have shown that certain mating types can often be associated with specific clinical manifestations, such as pulmonary or central nervous system infections.

# CONCLUSION AND RECOMMENDATION

In this research, the DNA of the Clinical isolate FBC and Environment isolate NBM5 have been extracted and isolated and in the near future, Polymerase chain reaction (PCR) analysis will be performed on them. The Clinical isolate FBC and Environment isolate NBM5 displayed similar responses when subjected to both antifungal susceptibility test and environmental stressors- thus confirming that the two strains are of the same mating type. This is an indication that clinical and environmental samples can be of the same mating type and virulence. The *Cryptococcus neoformans* is therefore a genetic tool for further research in the area of pathogenicity and virulence genes which will be useful in the discovery and production of antifungal drugs.

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