Pathogenesis of Oral Candidiasis

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ABSTRACT:
Candida albicans is considered as commensal oral flora and is one of the most common fungal pathogens in the oral cavity causing severe opportunistic infections. They are dimorphic in nature, existing in the relatively innocuous yeast form and the invasive hyphal form which is responsible for the pathogenesis of Candidal infection by promoting formation of fungal biofilms and tissue penetration. The organism also exhibits various other virulence factors which also contribute to the pathogenicity of the organism depending on the host response. This paper presents a review on the current knowledge of candida infection, addressing the issue of various virulence factors and their interaction with the host environment resulting in oral candidal infection.

Key words: Candida albicans, hyphae, virulence factors, adhesions

INTRODUCTION
Candida albicans, a commensal oral flora, is considered to be the primary etiological agent for oral candidiasis. Other species of Candida such as C. tropicalis, C. parapsilosis, C. krusei, C. guilliermondii etc may also be found intraorally but rarely cause disease.¹ These organisms colonize at multiple mucosal sites leading to infections ranging from superficial "oral thrush" to disseminated systemic lesions in immunocompromised individuals if the organisms are released into the bloodstream.² The spectrum of oral lesions of candidiasis varies from large white plaques of pseudomembranous candidiasis on the buccal mucosa to erythematous lesions of chronic atrophic candidiasis on the palate.³

Candida albicans: A Dimorphic Fungus
Since C. albicans exists in the yeast form as well as in the hyphal form, they can be considered as dimorphic. However some authors consider it to be polymorphic exhibiting several different morphological forms including budding yeast cells (blastospores, blastoconidia), pseudohyphae (elongated yeast cells that appear as filamentous cell chains), true hyphae and chlamydospores.⁴ ⁵
A blastospore is a unicellular form of the fungus that divides by budding. A hypha is a long microscopic tube which comprises multiple fungal cell units divided by septa. Germ tube formation is the initial stage in yeast – hyphal transition. Mitosis takes place within the extending hypha and septae are formed at intervals within the hyphae. The entire fungal cellular aggregate including branching hyphae is termed as mycelium.6

Yeast – hyphal transformation takes place when temperature and pH alterations occur within the host. When the ambient host temperature is below 33°C and pH turns acidic (average pH = 4), the growth of yeast form is favored. Hyphal growth occurs when the temperature increases to around 37°C and pH turns neutral. Between these temperatures and pH values, pseudohyphae may be produced.3

These different morphologies of C. albicans have distinct roles in the course of infection. Saville et al 2003 stated that the yeast form is important for dissemination through the bloodstream and this was supported by Bendel et al 2003.7, 8 The yeast form also adheres to endothelial surfaces leading to colonization.9 The filamentous form is invasive and thus better adapted for invasion through the host epithelium.10 It has also been shown that the hyphal forms are more resistant to phagocytosis due to their morphology.4

Pathogenicity of Candida

Although Candida is seen as a commensal oral flora, the occurrence of candidiasis occurs in selective individuals predominantly those with compromised immune response or those with prostheses coupled with poor oral hygiene habits etc. The conversion of this relatively innocuous commensal organism to the pathogenic stage therefore depends on an interaction between the organisms with the host environment and the resident bacteria present and can be triggered by serum, proline, N-acetyl glucosamine, and different carbon sources etc.2, 4

Virulence Factors Expressed By C. albicans

In order to overcome host defenses and cause disease in an individual, various virulence factors are expressed by C. albicans. These depend on type of infection, site and stage of infection and nature of host response. Amongst the various virulence factors, hyphal formation phenotypic switching, adherence and production of extracellular hydrolytic enzymes have been most widely studied in recent years.22

Role of Hematophagous in Pathogenesis of Candida

Formation of hyphae results in the development of fungal biofilms which promotes adhesion to biotic or abiotic surfaces and tissue penetration leading to infection. These biofilms are relatively resistant to treatment with anti fungal agents and thus pose clinical problems.

Physical properties of Hyphae

1. Morphogenesis & Morphology:

Morphogenesis exposes surface molecules which are responsible for alerting the immune system to the presence of the pathogen. This is mediated by detection of pathogen associated molecular patterns (PAMP’s) and is microbe derived molecules that are recognized as non host by phagocytes. PAMP’s which are derived from fungi include cell wall polysaccharides (β glucans, chitin phosphomannan), fungal surface proteins, secreted fungal enzymes and their breakdown products and ATP released during host cell lysis.23-27
Pathogenesis of Oral Candidiasis

Grows in more than one morphological form - *Dimorphic*

**Factors influencing dimorphism**

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**Stage 1: Colonization**
Epithelial adhesion
Nutrient Acquisition

**Stage 2: Superficial Infection**
Epithelial penetration
Degradation of Host Proteins

**Stage 3: Deep seated Infection**
Tissue penetration
vascular invasion
Immune evasion or escape

**Stage 4: Disseminated Infection**
Endothelial Adhesion
Infection of other host tissues
Activation of coagulation cascade

**Virulence Factors:**

- Hypha Formation
- Adhesins
- Hydrolytic Enzymes

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- Host mimicry?
- Immunomodulators?

- Hypha Formation
- Adhesins
- Hydrolytic Enzymes
- Phenotypic Switching?
- Antioxidants?
- Immunomodulators?
Dectin 1 is a C-type lectin involved in recognition of \( \alpha \)-glucans found in cell walls of fungi resulting in production of cytokines which activate a group of proteins called the inflammasome, which is expressed within mucosal macrophages and dendritic cells. This inflammasome in turn releases interleukin IL-1\( \beta \) which recruits T cells and neutrophils which form the main line of defense against \( \text{C. albicans} \) via phagocytosis and deployment of neutrophil extracellular traps (NET's). Thus morphogenesis is a virulence factor if host immunity is compromised.

2. Direction Of Hyphal Tip

Directional growth is a function of polarized cells like fungal hyphae. The hyphae of \( \text{C. albicans} \) respond to various asymmetric environmental signals by altering their axes of growth in a regulated method.

Thigmotropism is the property by which hyphae of \( \text{C. albicans} \) change their direction of growth in response to alterations in surface topography. On hard surfaces or liquid media, the trajectory of hyphal growth is linear, while on semi-solid surfaces the hyphal tips grow in an oscillatory manner to form regular two dimensional sinusoidal curves and three dimensional helices. Galvanotropism is the property by which hyphae respond when exposed to electric field. The presence of calcium ions regulates these responses. The hyphal tip thus controls the direction of new growth in response to the environment and helps steer the hyphae towards the nutrients and also around obstacles.

3. Force and Adhesion

Along with directional growth, the hyphae also need mechanical forces to push the surrounding matrix in order to penetrate physical barriers and to steer around obstacles and towards nutrients. Candidal hyphae exhibit both these properties within the host. In order to exert adequate mechanical force for penetration of hyphae, the organism needs to adhere to the host surface.

\( \text{Hwp1} \) is a well known cell surface protein of \( \text{C. albicans} \) expressed only on the hyphal form. The protein \( \text{Hwp1p} \) is a substrate for host transglutaminases and thus it can be covalently cross linked to the surface of host tissue and result in adhesion of organism to the host. This was supported by the findings who conducted a study and inferred that \( \text{Hwp1} \) mutants are defective in adhesion to epithelial cells and fail to cause oral or esophageal lesions in immunodeficient mice. Thus suggesting that expression of \( \text{Hwp1p} \) adhesion on the surface of hyphae is important for epithelial penetration.

\( \text{Efg1p} \) dependent expression of appropriate adhesion allows tight adherence of \( \text{C. albicans} \) cells to epithelial surface, aiding the application of force by hyphae. Thus, components of the hyphal regulon act together to produce characteristic invasive lesions of mucosal candidiasis. Generation of adequate adhesion by developing hyphae is time dependent. Thus the number of cross links formed with the host increases with the hyphal length.

Stages in Candidal Infection

The progress of candidiasis can be divided into stages depending on the extent of invasion.

Stage 1: Colonization

Colonization of the oral cavity by \( \text{C. albicans} \) can be defined as the acquisition and maintenance of a stable population of \( \text{C. albicans} \) cells which does not give rise to clinical disease. Colonization depends on the rate acquisition- that is, the rate at which yeast cells enter the oral cavity - growth, and removal of cells from the mouth by swallowing and oral hygiene. To simplify this explanation, if the rate of removal is greater than that of acquisition and growth, clearance will take place. If the rate of removal is the same as that of acquisition and growth then there will be colonization. If the rate is lower and there is tissue damage, it will lead to candidiasis.

The presentation of candidiasis will depend on the tissue colonized, the virulence factors expressed by the Candida cells, and the host response. Thus, colonization depends on several factors like the acquisition or entry of cells into the oral cavity, the attachment and growth of those cells, the penetration of tissues, and the removal of cells from the oral cavity.

Stage 2: Superficial Infection by formation of Fungal Biofilm

Mucosal infections by \( \text{C. albicans} \) are caused primarily due to formation of biofilms where plaques formed by over-proliferation of adhered cells to oral epithelium are easily visible. After colonization of the yeast cells, a phase of cell filamentation and proliferation begins which results in formation of multiple layers of sessile cells of different
morphologies including pseudohyphal and hyphal cells. This is followed by maturation which results in a complex network of cells embedded in an extracellular polymeric matrix composed of carbohydrates, proteins, hexosamine, phosphorus and uronic acid.\textsuperscript{41}

There is also some evidence that host glycoproteins, nucleic acids and cells like neutrophils may also help in maturity of the matrix in particular on the mucosal sites.\textsuperscript{42-44} Hyphal formation is not essential for the formation of maintenance of biofilms, however, the tangle of hyphal filaments strengthens the structure of the biofilm.\textsuperscript{45}

**Stage 3: Tissue penetration**

Hyphae consist of a chain of elongated cells that expand at the apex of the cell tip.\textsuperscript{46} When cells divide, the mother and daughter cells remain confined to the particular site and thus compete for nutrients. Hyphal formation allows new cells to be produced subsequently by expansion at the tip. The bulk of the cytosol of the mother cell is pushed forward by turgor pressure along with expansion of the vacuoles positioned sub-apically.\textsuperscript{47} Thus, hyphal growth enables the organism to escape from a phagocyte, anchor within a cell layer or penetrate endothelia.\textsuperscript{2}

Hyphal growth is also accompanied by the secretion of exoenzymes which participate in lysis of the substrate or may be involved in synthesis of fungal cell wall.\textsuperscript{48, 49}

**Stage 4: Vascular Dissemination**

C. albicans is capable of causing bloodstream infections in humans which help in its translocation. Cells of C. albicans stimulate endothelial cells to take them up via endocytosis. Thus the uptake and subsequent damage of endothelial cells is promoted by hyphae and the expression of hyphal regulon.\textsuperscript{2} After being internalized, cells of C. albicans damage the endothelial cells, which in turn aids in more cells of C. albicans traversing the endothelium. The mechanism for endothelial damage is somewhat similar to that whereby the hyphae escape phagocytosis. Filamentous growth within endothelial cells may allow escape and transcytosis of the endothelial cell layer by C. albicans concomitant with endothelial cell damage.\textsuperscript{46}

**OTHER VIRULENCE FACTORS**

1. **Adherance & Adhesins**

   The persistence of candida on mucosal surface requires fungal adhesion to epithelial cells. Candidal adhesion is a complex process and the following three types of interactions have been proposed:\textsuperscript{50}
   
   1. Protein – Protein interactions in which a protein on Candidal surface recognizes a protein or peptide ligand on epithelial or endothelial cells
   2. Lectin like interactions in which lectin like proteins present on the Candidal cell wall adheres terminal sugars of the cell surface glycoproteins of human host\textsuperscript{51}
   3. Incompletely defined interactions in which a known surface component of C. albicans attaches to epithelial or endothelial surfaces by an as yet unidentified ligand

   Most of the adhesions identified to date are mannoprotein and for individual adhesins, both protein and carbohydrate portions have been implicated in adherence.\textsuperscript{40}

2. **Phenotypic Switching:**

   Besides the dimorphic transition from yeast to hyphae, C. albicans is also capable of a different form of morphologic change known as ‘phenotypic switching’ which is readily observed in the morphology of colonies.\textsuperscript{52, 53}

   Two examples of phenotypic switching have been described in C. albicans: the white–opaque transition and 3153A-type switching. In the white–opaque transition, first described in the strain WO-1, cells switch between a form that gives rise to hemispherical, creamy white colonies (white phase) and a form that produces flat, gray colonies (opaque phase).\textsuperscript{54, 55} The difference between white and opaque colonies reflects a dramatic difference in the appearance of individual cells. While cells in a white colony are relatively round (comparable in shape to those of S.cerevisiae cells), those isolated from an opaque colony are elongated, asymmetrical and show surface pimples.\textsuperscript{53}

   The other switching system, 3153A-type switching, was described in cultures of the standard laboratory strain 3153A, and it has also been observed in other laboratory strains and clinical isolates.\textsuperscript{56-58} Distinct from the white– opaque transition, this type of switching produces at least seven different colony morphologies. The predominant colony type in this phenotypic strain is ‘smooth’, but variant colonies arise at a frequency.
of $\sim 10^{-4}$. These variant colony morphologies reflect, at least in part, the combination of the three main cellular forms of *C. albicans* cells (blastospores, pseudo-hyphal and hyphal cells) found in the colony dome, although the exact relationship between the colony morphology and the cellular forms that comprise it is not known.56, 59

Switching can be triggered by low doses of UV radiation and after being triggered into the 'high frequency switching mode', *C. albicans* exhibits high rates of alteration in colony morphology.3

The extraordinarily high frequency and reversibility of switching, the distinct phenotypes in the two switching systems, the developmental differences in hyphal formation and the differences in sensitivity to antifungal agents all suggest that switching systems have a role in pathogenesis. The pathogenic mechanisms of *Candida* which may be potentiated by switching mechanisms include the capacity to:

1. Invade and proliferate in extremely different body environments
2. Elude immune system by alterations in surface antigenicity

Switching may also selectively enhance the adhesion to oral mucosa, tissue penetration and secretion of enzymes such as proteinases and phospholipases.3

3. **Extracellular Hydrolytic Enzymes**

In susceptible hosts, *C. albicans* enters the bloodstream and causes deep seated infection in target organs. One factor that contributes to the process of virulence is hydrolytic enzyme production, which is known to play a central role in the pathogenicity of *C. albicans*. Two types of secreted enzyme seem to be the most important: phospholipases and secreted aspartyl proteinases.60, 61

**Secreted Aspartyl Proteinases**

The extracellular proteolytic activity of *C. albicans* was discovered by Staib (1965), who was also the first to suggest a role for candid proteinases in the pathogenesis of candidiasis.3 Evidence of a role of candida proteinase in the pathogenesis of candidiasis was briefly reviewed by Odds (1985).3 In a study done by MacDonals and Odds (1983), it was found that chemically induces mutant strains of *C. albicans*, which showed low proteolytic activity were less virulent in mice than the strongly proteolytic parental strains. These results were confirmed by Kwon – Chung et al (1985) and they also showed that revertants with restored proteolytic activity also regained their virulence.3

Secreted Aspartyl proteinases are known to degrade many human proteins, including mucin, extracellular matrix proteins, numerous immune system molecules, endothelial cell proteins, and coagulation and clotting factors. Therefore, the action of Sap proteins could be involved in all four stages of infection and probably greatly enhances the pathogenic ability of *C. albicans*.22

**Lipases:**

Lipolytic activity of *Candida* species was first described by Werner (1966), who recognized such activity amongst strains of *C. albicans*, *C. stellatoidea* and *C. tropicalis*. The lipolytic activity of *C. albicans* was differentiated into phospholipase A and lysophospholipase by Price and Cawson1977. Phospholipase production in *C. albicans* is limited to acidic growth conditions (ph 3.6 – 4.7) and is inversely proportional to the concentration of glucose and galactose in the media.3 Phospholipases are most likely to contribute to the pathogenicity of *C. albicans* by damaging host-cell membranes, which aids the fungus by facilitating invasion of host tissues.62

**Conclusion**

*Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for *Candida* species to invade tissues normally resistant to invasion.

The increased prevalence of local and systemic disease caused by *Candida* species has resulted in numerous new clinical syndromes, the expression of which depends primarily on the immune status of the host. The conversion of this relatively innocuous commensal organism to the pathogenic stage depends on an interaction between the organisms with the host environment and the resident bacteria present. *Candida* species also exhibit their own set of well-recognized virulence factors that may contribute to their ability to cause infection.
Thus the exact nature of determinants of virulence in candida and the response of the host to these different virulence factors needs to be investigated further using recent advances in technology.

REFERENCES:


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