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The role of virulence factors in *Candida albicans* pathogenicity

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ABSTRACT

Candida albicans is a classical example of causative agent for opportunistic fungal infection. Normally, it colonizes skin, gastrointestinal tract, genital, and mucosal membranes, but in certain condition it may responsible for diseases. This phenomenon was mainly associated with immunological status of the host. However, there were findings that showed the possibility of putative virulence factors work on the transition of commensally to pathogenic role of the yeast. In this review, some virulence factors were discussed. Indeed, there were factors that may be considered as putative virulence factors of *C. albicans*.

ABSTRAK

Candida albicans adalah contoh klasik jamur penyebab infeksi oportunistik. Jamur ini biasa berkoloni di kulit, traktus gastrointestinal, genital, dan membran mukosa sebagai komensal. Namun demikian, pada kondisi tertentu jamur ini dapat menjadi agen penyebab infeksi. Fenomena ini biasanya dihubungkan dengan status imunitas dari inang. Akan tetapi, terdapat banyak laporan yang mengindikasikan adanya faktor virulensi yang bekerja pada proses transisi dari komensal menjadi patogenik ini. Pada kajian pustaka ini, beberapa kandidat faktor virulensi *C. albicans* didiskusikan.

Keywords: *Candida albicans* – opportunistic infection – fungal – pathogenicity - virulence

INTRODUCTION

Candida albicans is a classical example of causative agent for opportunistic fungal infection. There are 17 species member of genus *Candida* e.g: *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* which are commonly related with human diseases.¹ The clinical manifestations of *C. albicans* infection are related with the host immune response. The risk of infection is higher in the individual with risk factors related with immune

response deterioration. It is well known that *C. albicans* is a flora normal in the skin, gastrointestinal tract, genital, and mucosal membranes, which is not regularly recognized as a pathogen in immunocompetence human being.² *Candida albicans* may be cultured on several media, such as Sabouraud's dextrose agar. It grow very fast, unlike other known fungus. The yeast colony is white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like cells or blastoconidia, 2.0-7.0 x 3.0-8.5 um in size. Assimilation test showed

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positive for: Glucose, Maltose, Galactose, Trehalose, Sucrose (some negative), D-Xylose, Soluble Starch, D-Mannitol, and D-Glucitol (Delayed).³ *Candida albicans* may be identified using several methods, i.e. microscopic, morphological observation on selective media, germ tube test, assimilation test, and nucleic acid base identification (FIGURE 1).



FIGURE 1. Colony appearance of *C. albicans* isolated from sputum which was cultured on Sabouraud's dextrose agar media, after incubation in room temperature for 24 hours. The yeast colony appeared white to cream colored, smooth, glabrous and yeast-like in appearance.

Candida albicans is the most common fungal causative agent for urinary tract infection. In general, *C. albicans* is unusual cause of urinary tract infection in healthy individual. However, it was reported common in hospitalized patients, who have risk factors, such as urinary tract structural problems. The infection may associate with invasion in antegrade form blood stream or retrograde from urethra and bladder.⁴

A surveillance of nosocomial blood stream infections (BSI) report in the USA between April 1995 and June 1996 highlighted that *Candida* was the fourth most common causative agent of nosocomial BSI. Fifty-

two percent of 379 of candidemia cases were due to *C. albicans*,⁵ and it have reported to be one of the leading causes of catheter-related BSI.⁶ Candidemia affects more than 250,000 people worldwide every year and responsible to more than 50,000 deaths. Incidence rates of candidemia have been reported to be 2 - 14 cases per 100,000 persons in population-based studies.⁷

Epidemiological data showed that *Candida* may cause wide spread of vulvovaginal candidiasis (VVC). VVC is most frequently caused by *C. albicans*, though other species are emerge.⁸ This fungal infection affect up to 75% healthy women, which some of them develop recurrent infection, known as recurrent vulvovaginal candidiasis (RVVC). The RVVC has challenging characteristic which may responsible for treatment failure.⁹ Many factors work to facilitate the transtition of *C. albicans* colonization state to asymptomatic fungal infection, such as host susceptibility and host inflammatory responses, as well as candidal virulence factors.^{10,11}

Candida sp are members of the oral microflora of humans, they are opportunistic pathogens that under conditions of host debilitation can cause an oral infections. Alteration of oral environment may favor the overgrowth of *Candida*, which in turn develop oral candidiasis. *Candida sp* is well known as pathogen responsible for oral diseases.¹² In oral fungal infection, *C. albicans* contributes near 50% of all candidiasis cases.¹³

Candidiasis (moniliasis) is the third most common superficial skin fungal infection after dermatophytosis and pityriasis versicolor.¹⁴ Non-hematogenous primary skin infections typically occur as intertrigo in skin folds, which is common in obese and diabetic patients. *Candida* is known responsible for neonatal candidiasis syndrome, a widespread dermatitis due to *Candida* in neonates. The

syndromes associated with contamination of amniotic fluid, in which limited to the skin for healthy-term infants.¹⁵

There were other various systems and organs may be involved for *C. albicans* infection, such as: liver, spleen, larynx, lungs, bones, joints, pancreas, peritoneum, endocardium, eyes, and meninges.¹⁵ The *C. albicans* clinical manifestation is considered as transformation from commensal of the fungi to infection state. There are many factors work for the transformation, including virulence factors of *C. albicans*. This review aims to discuss several putative virulence factors of *C. albicans*, which is important to give more understanding and insight concerning to the management of candidiasis which may involve various organs.

DISCUSSION

There are various putative virulence factors of *C. albicans* documented, such as: adherence of the fungi to the host's surfaces, production of hydrolytic enzymes, dimorphism, galvanotropism and thigmotropism, phenotypic switching, biofilm formation, and evasion to the host immune response.

Adherence

Candida albicans is able to adhere to various tissues and inanimate surfaces. For example, buccal and vaginal epithelial cells, corneocytes, cultured cells (HeLa and HEp-2) surface, as well as biomaterial surface.⁴ Adherence is the first step of *C. albicans* infection in the oral and other surfaces. It is an essential stage for the persistence of the organism in the host, as shown by the report that number of *C. albicans* cells adhered to epithelial cells is significantly higher in the chronic periodontitis group

than in the control group.¹⁶ Experimentation using saliva – coated hydroxyapatite (SHA) beads showed that *C. albicans* strains which associated with oral candidiasis adhere better to SHA beads than less pathogenic strains.^{17,18} Adhesins are the fungal surface molecules that mediate binding of *C. albicans* to the surface of human or microbes cells, inert polymers, or proteins.¹⁹ There are candidate genes putatively considered as encoding adhesins such as: *ALAI*, *ALSI*, *Hwp1*, *INT1*, *MMT1*, *PMT1*, *PMT6* and *Als1p*.¹⁸⁻²¹ Other putative adhesins are mannan, chitin, factor 6 oligomannosaccharide, 66-kDa fimbrial protein, fibronectin binding protein, iC3b binding protein, fucose binding protein, GlcNAc or glucosamine, and secreted aspartyl proteinase (SAP).^{18,19} Efforts to understand *C. albicans* adhesins are hampered by the limitation of consistency in several studies, such as: different strain showed different adherence specificities and strength, technical limitation of maintaining adhesins expression *in vitro*, and disagreement of viability yeast effect to the adherence capability.¹⁸

Production of hydrolytic enzymes

Candida albicans is producing many hydrolytic enzymes that facilitate its commensally and pathogenic characteristics: adherence to host tissue and inert particles, rupture of host cell membranes, invasion of mucosal surfaces and blood vessels, and evasion of the host's immune response. There are three major enzymes produced by *C. albicans*: SAP, phospholipases, and hemolysins.^{4,22}

Secreted aspartyl proteinases

The Sapropeins of *C. albicans* were encoded by a family of 10 *SAP* genes: *SAP1*, *SAP2*, *SAP3*, *SAP4*, *SAP5*, *SAP6*, *SAP7*, *SAP8*,

SAP9, and *SAP10*. The Sap1 to Sap10 proteins are 35- 50 kDa in size and responsible for all of the extracellular proteolytic activity of *C. albicans*. The characteristic of Sap proteins are not yet clearly elucidated, though current evidences showed that the main roles of the *C. albicans* Saps are to provide nutrition for the yeast cells, to aid penetration and invasion, and to evade host immune responses.²³ Sap degrades proteins related to structural and immunologic defenses, such as collagen, keratin, mucin, antibodies, complement, and cytokines, during tissue invasion.^{4,24} The presence of genes and secretion of aspartic proteases by *C. albicans* was demonstrated to be one of the virulence factors.²⁵ It was observed that the virulence of *C. albicans* associated with the level of Sap activity and number of *SAP* genes. *C. albicans* isolated from patients who has clinical manifestation, have higher proteolytic activity compare to those obtained from healthy individual.^{23,26,27} Saps expression by *C. albicans* is regulated by several factors, such as nutritional condition, pH, temperature, and growth phase of the yeast. Studies on Saps expression revealed that there are contradiction conclusions on Sap expression in various *in vivo* experiments. Sap protein specific types is claimed to be correlated with organ involve, infection locataion, and environmental factors in every study. However, because of different techniques and experimental model, effort to formulate conclusive statement on which type of Sap dominantly active in each candidiasis cases -- for example which Saps work in oral candidiasis, vaginal candidiasis, or systemic candidiasis -- is a very difficult task.²⁸

Phospholipases

Phospholipases hydrolyze glycerophospholipids, which are major components of mammalian cell membranes. It cleaves fatty acids from phospholipids which in

turn destabilizing the membranes.^{4,29,30} There are seven phospholipase genes have been identified i.e. *PLA*, *PLB1*, *PLB2*, *PLC1*, *PLC2*, *PLC3* and *PLD1*. However, the role of the enzymes encoded by these genes remains unclear.³¹ Evidence of phospholipase act as virulence factor was obtained from comparative study conducted by Ibrahim *et al.*³² in which one series of *C. albicans* obtained from candidemia patients compared with isolates obtained from oral cavity of healthy individuals. It was shown that isolate from candidemia cases have higher phospholipase activity, which reflected the virulence of these isolates.

Hemolysins

Hemolytic capacity, which is served by hemolysins enzyme, is an important putative virulence factor of the genus *Candida* to acquire iron from host tissues, which then is used by the yeast for metabolism, growth and invasion during host infection.^{33,34} In the human being, iron is found in several proteins, including hemoglobin located in the erythrocytes. *Candida albicans* capable to bind to erythrocytes, then hemolysis factor will destroy the erythrocytes. However, the mechanism of hemolysis caused by *C. albicans* remain unclear.^{33,34} The hemolytic activity may be observed readily by culturing *C. albicans* on the blood agar media.

It was reported that 98.5% of *C. albicans* isolates have hemolytic activity including alpha, beta, and gamma hemolysis in aerobic condition.³⁵ However, the hypothesis of hemolysins is a virulence factor of *C. albicans* still controversial. Study on *C. albicans* isolated from HIV patients and healthy individual showed that the hemolytic activity were higher in isolates which were obtained from healthy individual.³⁶ This finding add another insight that hemolytic capacity may not be considered as virulence

factor without presence of other virulence factors.

Dimorphism

Candida albicans able to grow in yeast and mold forms. The transition between yeast and hyphal forms is termed as dimorphism. Accordingly it is classified as a dimorphic fungus. In the yeast form, it may undergo budding, whereas in the mould form, it may produce new mycelia, or yeast-like form. The transformation between two morphologies can be induced *in vitro* with several environmental conditions, such as pH, temperature, or chemicals.³⁷ Dimorphism of *C. albicans* is a unique characteristic for the pathogenicity of the yeast (FIGURE 2). Both morphologies have their own function to support its virulence.³⁸ The hyphal form has been reported to be more invasive than the yeast form. It was shown that three *C. albicans* mutant strains which compromised in the ability to form hyphae (*efg1Δ/efg1Δ*, *flo8Δ/flo8Δ*, and *cph1Δ/cph1Δ efg1Δ/efg1Δ*) were significantly less virulent in *C. elegans* infection model.³⁹ Whereas, the yeast form is proposed as the form primarily involved in dissemination of the fungus.⁴⁰ However, another group found that transition of *C. albicans* morphology to yeast form may not be the only factor that regulates dissemination from the gastrointestinal tract to the other organs in invasive *C. albicans* infection.⁴²

More than 40 genes were identified responsible for dimorphism regulation, mainly hyphal formation.^{42,43} Those genes are working in different stages of infection. Dimorphism of *C. albicans* is important for pathogenicity at both superficial and systemic infections. It should be noted that both yeast and filamentous forms of *C. albicans* were found in infected tissues. The capability of *C. albicans* to undergo transition from yeast to filamentous form contributes to the numerous nature of its infection stages, such

as adherence to epithelial and endothelial cells, intercellular invasion, iron acquisition from intracellular host sources, biofilm formation, as well as escape from phagocytes and immune evasion.^{44,45}

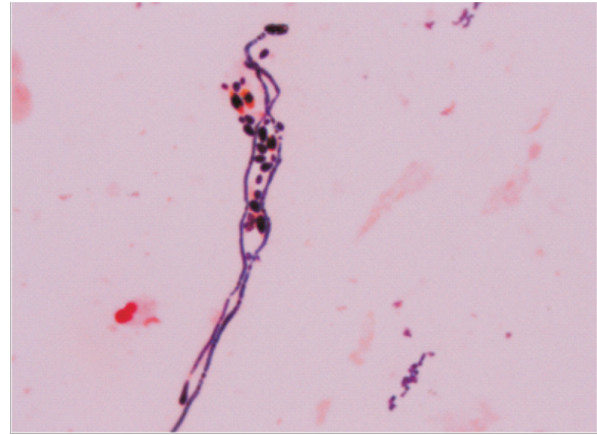


FIGURE 2. Microscopic appearance of *C. albicans* found in the sputum of patient, after Gram staining. The dimorphism of *C. albicans* budding yeast, pseudohyphae, and true hyphae were observed.

Phenotypic switching

Candida albicans has the capability to undergo phenotypic switching that is commonly called as white-opaque phenotypic switching. A small proportion of *C. albicans* isolates, which are homozygous at the mating type locus (*MTL*, *a/a* or *α/α*), are able to switch between two distinct cell morphologies: white and opaque. It is not known how white-opaque switching has never been observed in *C. albicans* strains that have heterozygous *MTL* genotypes (*a/α*), though they have all essential genes for white-opaque switching.⁴⁶ FIGURE 3 shows different cell morphology between white and opaque cells. The white cells appear round and bright under the microscope, while opaque cells appear darker, polymorphic and oval. The white-opaque cells may be observed also in their colonies by using simple phloxin B supplementation in the media.³⁶ Furthermore,

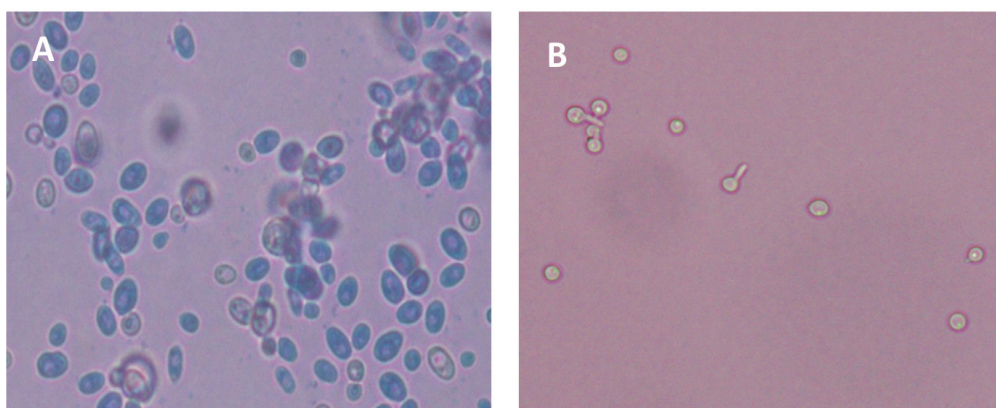


FIGURE 3. Microscopic appearance of *C. albicans*. (A) Opaque cells appear dark, oval and polymorphic. (B) White cells appear circular and bright. Some of them produce hyphae when incubated in 37°C for 90 minutes with supplementation of serum (germ tube test).

white and opaque phenotypes show different cellular and colony appearances, gene expression profiles, mating ability and virulence.⁴⁷

White and opaque cells differ in their mating capability as well as expression of genes that are unrelated with mating process, such as adhesins and metabolic genes. Opaque cells are better colonizers of the skin, but they are less virulent than white cells in a mouse model of disseminated candidiasis.⁴⁸ Opaque cell formed hyphae in very low level in the suspension cultures, in which white cell *C. albicans* will able to form hyphae. This finding indicated that opaque cell less virulent compare to white cells. When opaque cells able to form hyphae, morphologically similar with hyphae of white cells. However, genetically still distinct to the hyphae formed by white cells.⁴⁹ White-opaque switching occurs at a low frequency in *C. albicans*,³⁶ but certain environmental conditions can drive the switch from one phase to the other. Although opaque cells are less frequently cause systemic infection than white cells, they have better optimization for colonization, such as on the skin.⁵⁰ White-opaque switching has been

shown also to affect several virulence factors, such as susceptibility to antifungal drugs, proteinase activity, antigenicity, and adhesion of *C. albicans*.⁴

Biofilm formation

Biofilm is a structure made of microbes consortium supported with extracellular matrix which attach to the surface of living matter or inanimate structure. *Candida albicans* is well known yeast that able to develop biofilm. Biofilm of *C. albicans* is notorious because of its deleterious consequences, such as leads to antifungal resistance, give a asylum to the yeast because of ability to make evasion against immune surveillance, and act as perfect reservoir for source of infection, as well as several advantages in the fungal's perspective: protection from the environment, resistance of physical and chemical stress, metabolic cooperation, and a community-based regulation of gene expression. Indeed, biofilm formation represents one of the putative virulence factors contributing to the pathogenesis of candidiasis.⁵¹⁻⁵³ The biofilm of *C. albicans* may need special attention since antifungal may not work properly in

the biofilm setting. Anti-biofilm substance need to be invented in the near future.⁵⁴ Biofilm formation is a dynamic process which is started with adherence of planctonic yeast cells to the surface, proliferation of the yeast cells, formation of hyphae, and accumulation of extracellular matrix. Then the maturation of the biofilm is complete. Moreover, yeast cells which construct the biofilm may be detached and disperse to other part of the body which may go to the new focal infection.^{51,55} There are two types of *C. albicans* cells involve in the biofilm formation: small yeast-form cells (blastospores), and long tubular hyphal cells. The two cell types have their specific role in biofilm formation.⁵⁵ Study on two series *C. albicans* from HIV infected and healthy individuals showed that frequency of *C. albicans* isolates which able to form biofilm is comparable between two groups.³⁶ The result showed to us that biofilm as a putative virulence factor may not work alone. It may work in a concert with other virulence factors.

Evasion to the host immune responses

Human immune response against *C. albicans* occurs through several mechanisms, comprising innate and adaptive immune response. The innate immune response is nonspecific and broad. This is the first line of host defense against potentially harmful microbes. Innate immunity comprises of a group of soluble (complement) and cellular (neutrophil, macrophage) components. Whereas, the adaptive immune response recognizes specific antigenic moieties, resulting to the development of a targeted immuneresponse.⁵⁶ Several mechanisms have been proposed to explain the mechanism of *C. albicans* evade from the host immune response, which is considered as virulence factor of the yeast. Experiment in *TLR2*(-/-) mice showed that *C. albicans* induce

immunosuppression through TLR2-mediated IL-10 release, and this leads to generation of CD4+CD25+ T-regulatory cells with immunosuppressive potential.⁵⁷ *Candida albicans* was shown capable to bind thrombocytes via fibrinogen ligands in the blood stream. This may result to the yeast cells being surrounded by a group of thrombocytes, which in turn may camouflage them from the immune system during dissemination through the blood stream.⁴

CONCLUSION

Candida albicans is an opportunistic pathogen which responsible for various diseases associated with several organs. Its pathogenicity is not solely because of the impairment of host immune response. Some putative virulence factors may work in concert to facilitate the yeast transition from commensal to pathogenic. Now, it is important to further analyze the role of each virulence factors in every step of the transition, in order to improve the management of the candidiasis in the near future.

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