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Genetic determinants of virulence – *Candida parapsilosis*

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ABSTRACT

The global epidemiology of fungal infections is changing. While overall, *Candida albicans* remains the most common pathogen; several institutions in Europe, Asia and South America have reported the rapid emergence to predominance of *Candida parapsilosis.* This mini-review examines the impact of gene deletions achieved in *C. parapsilosis* that have been published to date. The molecular approaches to gene disruption in *C. parapsilosis* and the molecularly characterized genes to date are reviewed. Similar to *C. albicans*, factors influencing virulence in *C. parapsilosis* include adherence, biofilm formation, lipid metabolism, and secretion of hydrolytic enzymes such as lipases, phospholipases and secreted aspartyl proteinases. Development of a targeted gene deletion method has enabled the identification of several unique aspects of *C. parapsilosis* genes that play a role in host–pathogen interactions – CpLIP1, CpLIP2, SAPP1a, SAPP1b, BCR1, RBT1, CpFAS2, OLE1, FIT-2.

This manuscript is part of the series of works presented at the "V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi" (Oaxaca, Mexico, 2012).

Determinantes genéticos de la virulencia de *Candida parapsilosis*

Palabras clave:
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Alteración génica
Lipasa
Secreción de aspartilproteinasas
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RESUMEN

La epidemiología mundial de las infecciones fúngicas está cambiando. Aunque *Candida albicans* sigue siendo el patógeno más común, varios centros en Europa, Asia y Sudamérica han descrito la rápida emergencia de *Candida parapsilosis*, que ha terminado por predominar. La presente revisión examina la influencia de las deleciones genéticas producidas en *C. parapsilosis* que se han publicado hasta la fecha. Se revisan las estrategias moleculares de la alteración de genes de *C. parapsilosis* y los genes caracterizados molecularmente hasta la fecha. Al igual que en *C. albicans*, los factores que influyen en la virulencia de *C. parapsilosis* incluyen la adherencia, formación de biopelículas, el metabolismo de lípidos y la secreción de enzimas hidrolíticas, como lipasas, fosfolipasas y aspartilproteinasas. El desarrollo de un método de deleción génica dirigido ha permitido la identificación de varios aspectos exclusivos de los genes de *C. parapsilosis* que participan en las interacciones huésped-patógeno-CpLIP1, CpLIP2, SAPP1a, SAPP1b, BCR1, RBT1, CpFAS2, OLE1, FIT-2.

Este artículo forma parte de una serie de estudios presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

Since the late 1970s, fungal infections have increasingly become a significant cause of morbidity and mortality especially among hospitalized and immunosuppressed patients.[69] *Candida* species are the fourth most frequent causative agent of blood-stream infections, constituting 8–15% of hospital-acquired infections.[87] In the United States, *Candida albicans* is the most common pathogen followed by *Candida parapsilosis* or *Candida glabrata*, depending on the study. However, *C. parapsilosis* has become the leading causative agent in some institutions located in Europe, Asia and South America, and it is the *Candida* species with the largest increase in incidence since 1990.[1,6,10–12,20,36,48,52,54,70,74,81,87] Of all candidal isolates, *C. parapsilosis* accounts for 15.5% in North America, 16.3% in Europe and 23.4% in Latin America.[81] In the US, it has been the third most common cause of neonatal sepsis.[66]

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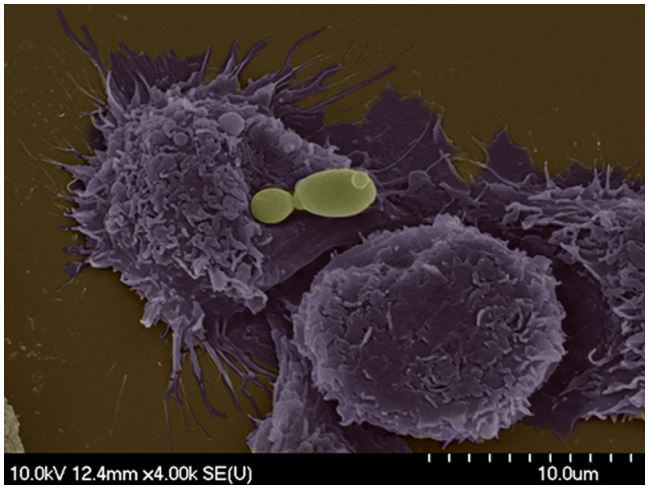

Fig. 1. Scanning electron microscopy of *C. parapsilosis* interacting with murine macrophages (photo credit to T. Németh, T. Petkovits, and A. Gácser).

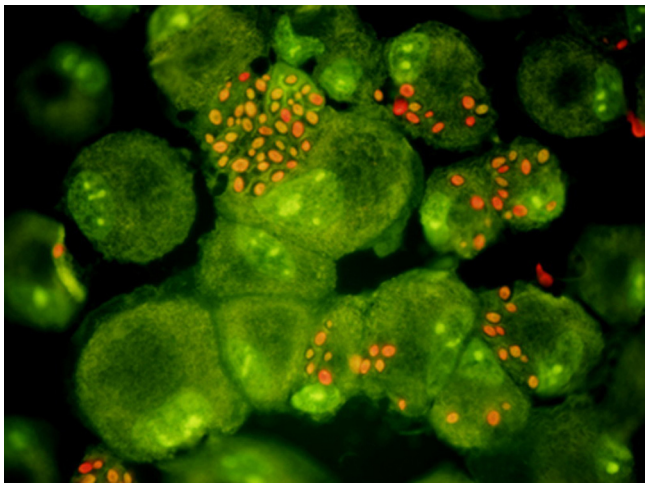

Fig. 2. Immunofluorescence microscopy of *C. parapsilosis* and human macrophages. Red yeast are dead (photo credit to C. Papp and A. Gácser).

First isolated in 1928 from a stool specimen and thought to be non-pathogenic,[2,86] *C. parapsilosis* is now recognized as being fairly ubiquitous as it can be isolated from humans as a normal skin commensal as well as from domestic animals, insects, soil and marine environments.[19,81,85] It is now especially well documented as a pathogen that arises from exogenous sources of infection in intravenous drug users and via medical instrumentation (e.g. catheters and hyperalimentation solutions).[34,81] In particular, *C. parapsilosis* is recognized for its ability to cause invasive disease in patients without prior evidence of colonization via horizontal transmission through medical devices including catheters, parenteral nutrition solutions, and the hands of healthcare workers.[81] Invasive disease occurs more often in immunocompromised patients, such as individuals with AIDS, cancer and in patients undergoing gastrointestinal surgical procedures.[81] Other risk factors reported in studies include transplant receipt, antibiotic exposure, ophthalmic irrigating solutions and, especially, low birth weight in premature neonates.[1,44,78,85,86] Clinical manifestations of *C. parapsilosis* include endocarditis, meningitis, peritonitis, arthritis, endophthalmitis, keratitis, otomycosis, onychomycosis, vulvovaginitis and urinary tract infections.[81] Mortality rates attributed to *C. parapsilosis* range from 4% to 45%, with an average mortality rate of 28.5%.[6,11,28,81] Biofilm producing isolates are associated with outbreaks[38] and significantly higher mortality rates.[82]

Determinants of virulence for candidal disease include adhesion capability to host surface, ability to switch morphology between yeast and filamentous growth, biofilm formation and secretion of extracellular hydrolytic enzymes such as lipases, phospholipases or secreted aspartyl proteinases.[51,83] Conflicting data exist regarding phospholipase activity, with some studies demonstrating their presence in clinical isolates and others their absence.[14,16,26,47] Nevertheless, its role in virulence bears consideration. The development of gene disruption methods to produce mutants has been a pivotal achievement in our capacity to gain insights into the interactions of *C. parapsilosis* with hosts and host effector cells (Figs. 1 and 2). This review will focus on the observations made on specific genes that have so far been characterized and shown to significantly influence these virulence traits.

Gene disruption

C. parapsilosis has a diploid genome and does not have a described sexual cycle. Genetic analysis was initially limited by the availability of appropriate study tools. The first targeted gene disruption method was developed in 2007[23] based on previously established gene disruption protocols in *C. albicans*. The first targeted deletion in *C. parapsilosis* was the disruption of secreted lipases. This efficient gene deletion system was developed utilizing the repeated use of the dominant nourseoethricin marker (caSAT1) and subsequent deletion by FLP-mediated, site-specific recombination.[23] Applying this technique, the lipase locus in *C. parapsilosis* containing the adjacent lipase genes *CpLIP1*, *CpLIP2* were deleted and *CPLIP2* reconstructed providing an understanding of the role of lipase activity in virulence.[23]

Lipases

Microbial extracellular lipases are virulence factors in a broad range of bacteria and fungi, including *Candida* species.[81] So far, 10 lipase genes have been identified in *C. albicans*,[32] and disruption, such as the deletion of *LIP8*, can significantly affect virulence.[22] Only two lipase genes have been elucidated in *C. parapsilosis* – *CpLIP1* and *CpLIP2*, of which only the latter has been demonstrated to code for an active protein.[7,53] Utilizing the described targeted gene deletion method, disruption of these lipase genes provides evidence that they play a role in pathogenesis (i) by the decreased tissue damage seen in the presence of lipase inhibitors, (ii) the decreased ability of *CpLIP1-CpLIP2* homozygous mutants to form complex biofilms, (iii) requirement for lipid-rich media, (iv) increased susceptibility to phagocytosis by macrophage-like cells, and (v) decreased virulence in comparison to wild-type *C. parapsilosis* yeast in infections of human oral epithelium or during murine intraperitoneal challenges.[21,23] These observations hold significant clinical relevance since *C. parapsilosis* infections are particularly more commonly seen in patients receiving lipid-rich total parenteral nutrition and thus lipases may be a potential target for future antifungal agent development.[81]

Secreted aspartyl proteinase (Sap)

Secreted aspartyl proteinase (Sap) genes have been demonstrated in most pathogenic *Candida* including *C. albicans, Candida dubliniensis*, *Candida tropicalis* and *C. parapsilosis*.[25,45,49,89] However, they are notably absent in many non-pathogenic yeasts (e.g. *Saccharomyces cerevisiae*) suggesting their possible role in virulence.[30] Sap isoenzymes have several functions such as (i) digestion of host proteins for provision of nitrogen sources, (ii) degradation of host cell surface structure and intracellular substances promoting tissue adhesion and invasion, and (iii)