Looking into the virulence of *Candida parapsilosis*

A diagnostic perspective

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In many diagnostic microbiology laboratories the species identification process starting from cultured colonies has recently undergone some significant changes. Where not long ago carbon/nitrogen source utilization tests in the form of colorful tubes or well-based devices down to chip card size dominated the field, we now find a purely biophysical instrument, a MALDI-ToF mass spectrometer. Instead of evaluating growth only after overnight incubation, the results are now available within minutes of sample processing. At the same time, the depth of differentiation is significantly increased beyond what a cultural method can do. In an assimilation assay the number of data points is limited by the number of compounds tested, in mass spectrometry by the number of biomarker ions observed. In the first, common numbers range between 16 and 42, many of these not being able to discriminate between closely related species. In the latter, the number of biomarker ions observed usually exceeds 100, depending on spectrum quality. These are spread out over a mass range of approximately 10 kDa and most of them are unique even between closely related species, to some degree even between different isolates of the same species.

It is not surprising that this new availability to easily type with this depth has led to increased observation of “rare” microorganisms in clinical specimen. This is not only true for bacteria, but also for fungi. Mainly four complexes of species occur in clinical specimen, that are now easily distinguished: (1) *Candida albicans/dubliniensis*, (2) *C. glabrata/nivarienisi/bracarensis*, (3) *C. parapsilosis/orthopsilosis/metapsilosis*, and (4) what was identified as *C. famata* (Debaryomyces hansenii) by biochemical assays is obviously more likely to be a strain of *C. palmioleophila* or *C. guillermondii*.9,7

Here the clinical microbiologist is currently faced with a dilemma. What do we tell a clinician to do about *C. palmioleophila* unless it came from a sterile site? Do we actually report “*C. metapsilosis*” or just a “*C. parapsilosis*” group isolate? Clearly, the answer to these questions warrants investigation of the pathogenic potential of these—as compared with the major pathogenic yeast *C. albicans*—less frequent species.

One of the traits shared by pathogenic *Candida* species, in contrast to their closely related apathogenic sibling species, is the assembly of large gene families which constitute potential virulence factors. Among these, probably the most prominent is the family of ten secretory aspartic proteases of *C. albicans*, which have been shown to display differential pH optima and host tissue, as well as morphology-dependent gene expression patterns, highlighting their adaptation to the various niches and conditions found during the infection process. Apart from investigations toward specific differences between *C. albicans* and *C. dubliniensis*, the *C. parapsilosis* group is currently the next best investigated complex. Recent comparative genome data of several isolates suggests that these three species have actually diverged earlier than *C. albicans* and *C. dubliniensis*. *C. parapsilosis* is a commensal of the human skin and mostly known because of its potent ability to form biofilms on indwelling devices such as central venous catheters. One of the factors that might make *C. parapsilosis* such a successful colonizer of catheter surfaces is a large family of potential adhesins found in the genome, which is expanded by 5 members as compared with other yeasts of the CTG clade. Also virulence has been found to differ between the three species, which roughly correlates to the number of observations in clinical specimen, with *C. parapsilosis sensu stricto* being most virulent, and *C. metapsilosis* least. Similarly, other extracellular hydrolytic enzymes such as lipases and phospholipases are enriched in pathogenic species. Secretory lipases have previously been demonstrated to be highly important in virulence of bacteria, as well as in fungi like the skin-dwelling *Malassezia* species or *Candida* yeasts.

In humans, one of the major lines of defense against pathogenic yeasts is formed by macrophages. Consequently, in this issue of *Virulence*, Toth et al. turn to the influence of secretory lipases of *C. parapsilosis* on survival and pathogenicity in this cell type. In a series of experiments using a lipase deficient gene deletion strain, the authors show that the secreted lipase activity of *C. parapsilosis* “promotes the survival of fungal cells in macrophages and mitigates the inflammatory response of the host, thereby interfering with the efficient clearing of the pathogen”. There are only two genes (*CpLIP1* and *CpLIP2*) coding for such secretory lipases in *C. parapsilosis*, making it an excellent model to study strains of pathogenic yeasts with abolished extracellular lipase activity. When challenged with primary human macrophages, a higher rate of phagosome–lysosome colocalization was
observed in the lip1/lip2 mutant, which consequently was killed more efficiently.

One of the possible functions of such enzymes could be the liberation of fatty acids from tissues to support fungal growth. Indeed, tissue destruction by C. parapsilosis is mediated in part by lipases, but also by secretory proteases. Inhibition of these enzymes reduced epithelial damage but not invasion.

Next to its ability to adhere to catheter plastic material, C. parapsilosis is also an important pathogen because it is common in sepsis of preterm infants and neonates. It is mostly absent from mature children and older patients with a fully developed immune system. This points toward a direct interaction of C. parapsilosis with cells of the immune system in vivo. Indeed, the authors find patterns of cytokine expression (e.g., IL10) that suggest that secretory lipases might actually have anti-inflammatory potential, and might—directly or indirectly—work on regulatory immune lipids like prostaglandin or leukotrienes.

In C. albicans, which harbors a lipase family of ten members, such a gene deletion-driven study would not have been possible today. In earlier studies, the group tested the lip1/lip2 mutant strain and a collection of lipase-negative C. parapsilosis sensu stricto as well as sensu lato clinical isolates in other models. Here a similar pattern emerged: lipase negative isolates were generally less virulent and more prone to killing by macrophages.

It is noteworthy, that among C. parapsilosis clinical isolates, also in vitro extracellular lipase activity negative strains exist, while this is apparently not the case for in vitro extracellular protease activity. On a genome-sequence level, a recent analysis of several clinical C. parapsilosis isolates showed significant intra-species variability of at least ALS-family adhesin genes. Taken together, this may eventually lead to the discrimination of strains with higher and lower pathogenic potential in C. parapsilosis. Intriguingly, such diversity has been described for C. albicans and C. dublinensis. Using clinical isolates of different origin, infection experiments with a murine model of systemic candidiasis revealed striking differences in virulence. These ranged from total avirulence to full virulence in both species.

In a diagnostic environment, we can now ask if we can put this information to use. In fact, some advances using MALDI-TOF mass spectrometry for typing in fungi have recently been made by simple clustering of mass spectra. This also includes C. parapsilosis, where this technology has been used to track nosocomial spread of this fungus. In the future, this may be extended to predict isolates with relevant virulence phenotypes and highlights the importance to further study virulence phenotypes in these species and their occurrence in clinical isolates.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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