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.[1] 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] (2) *C. glabrata/nivariensis/bracarensis*,[3,4] (3) *C. parapsilosis/orthopsilosis/metapsilosis*,[3,5] 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. guilliermondii*.[6,7]

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.[8] 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.[9] Apart from investigations toward specific differences between *C. albicans* and *C. dubliniensis*,[10,11] 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*.[12] *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.[13] 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.[14] Similarly, other extracellular hydrolytic enzymes such as lipases and phospholipases are enriched in pathogenic species.[8] 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[15] or *Candida* yeasts.[9,16]

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".[17] 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 *lip/lip* 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.[16] 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.[18,19]

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.[20] 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,[21] 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.[14,22] 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.[14] On a genome-sequence level, a recent analysis of several clinical *C. parapsilosis* isolates showed significant intraspecies variability of at least *ALS*-family adhesin genes.[12] 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. dubliniensis*. Using clinical isolates of different origin, infection experiments with a murine model of systemic candidiasis revealed striking differences in virulence.[23] 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.[24] 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.

References

1. Bader O. MALDI-TOF-MS-based species identification and typing approaches in medical mycology. Proteomics 2013; 13:788-99; PMID:23281257; http://dx.doi.org/10.1002/pmic.201200468

2. Hof H, Eigner U, Maier T, Staib P. Differentiation of *Candida dubliniensis* from *Candida albicans* by means of MALDI-TOF mass spectrometry. Clin Lab 2012; 58:927-31; PMID:23163108

3. Pinto A, Halliday C, Zahra M, van Hal S, Olma T, Maszewska K, Iredell JR, Meyer W, Chen SC. Matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of yeasts is contingent on robust reference spectra. PLoS One 2011; 6:e25712; PMID:22022438; http://dx.doi.org/10.1371/journal.pone.0025712

4. Santos C, Lima N, Sampaio P, Pais C. Matrix-assisted laser desorption/ionization time-of-flight intact cell mass spectrometry to detect emerging pathogenic *Candida* species. Diagn Microbiol Infect Dis 2011; 71:304-8; PMID:21855250; http://dx.doi.org/10.1016/j.diagmicrobio.2011.07.002

5. Quiles-Melero I, García-Rodríguez J, Gómez-López A, Mingorance J. Evaluation of matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry for identification of *Candida parapsilosis, C. orthopsilosis* and *C. metapsilosis*. Eur J Clin Microbiol Infect Dis 2012; 31:67-71; PMID:21547602; http://dx.doi.org/10.1007/s10096-011-1277-z

6. Desnos-Ollivier M, Ragon M, Robert V, Raoux D, Gantier JC, Dromer F. *Debaryomyces hansenii* (*Candida famata*), a rare human fungal pathogen often misidentified as *Pichia guilliermondii* (*Candida guilliermondii*). J Clin Microbiol 2008; 46:3237-42; PMID:18701668; http://dx.doi.org/10.1128/JCM.01451-08

7. Castanheira M, Woosley LN, Diekema DJ, Jones RN, Pfaller MA. *Candida guilliermondii* and other species of candida misidentified as *Candida famata*: assessment by vitek 2, DNA sequencing analysis, and matrix-assisted laser desorption ionization-time of flight mass spectrometry in two global antifungal surveillance programs. J Clin Microbiol 2013; 51:117-24; PMID:23100350; http://dx.doi.org/10.1128/JCM.01686-12

8. Butler G, Rasmussen MD, Lin MF, Santos MA, Sakthikumar S, Munro CA, Rheinbay E, Grabherr M, Forche A, Reedy JL, et al. Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. Nature 2009; 459:657-62; PMID:19465905; http://dx.doi.org/10.1038/nature08064

9. Schaller M, Borelli C, Korting HC, Hube B. Hydrolytic enzymes as virulence factors of *Candida albicans*. Mycoses 2005; 48:365-77; PMID:16262871; http://dx.doi.org/10.1111/j.1439-0507.2005.01165.x

10. Moran G, Stokes C, Thewes S, Hube B, Coleman DC, Sullivan D. Comparative genomics using *Candida albicans* DNA microarrays reveals absence and divergence of virulence-associated genes in *Candida dubliniensis*. Microbiology 2004; 150:3363-82; PMID:15470115; http://dx.doi.org/10.1099/mic.0.27221-0

11. Jackson AP, Gamble JA, Yeomans T, Moran GP, Saunders D, Harris D, Aslett M, Barrell JF, Butler G, Citiulo F, et al. Comparative genomics of the fungal pathogens *Candida dubliniensis* and *Candida albicans*. Genome Res 2009; 19:2231-44; PMID:19745113; http://dx.doi.org/10.1101/gr.097501.109

12. Pryszcz LP, Németh T, Gácser A, Gabaldón T. Unexpected genomic variability in clinical and environmental strains of the pathogenic yeast *Candida parapsilosis*. Genome Biol Evol 2013; 5:2382-92; PMID:24259314; http://dx.doi.org/10.1093/gbe/evt185

13. Riccombeni A, Vidanes G, Proux-Wéra E, Wolfe KH, Butler G. Sequence and analysis of the genome of the pathogenic yeast *Candida orthopsilosis*. PLoS One 2012; 7:e35750; PMID:22563396; http://dx.doi.org/10.1371/journal.pone.0035750

14. Németh T, Tóth A, Szenzenstein J, Horváth P, Nosanchuk JD, Grózer Z, Tóth R, Papp C, Hamari Z, Vágvölgyi C, et al. Characterization of virulence properties in the *C. parapsilosis* sensu lato species. PLoS One 2013; 8:e68704; PMID:23874732; http://dx.doi.org/10.1371/journal.pone.0068704

15. Lee YW, Lee SY, Lee Y, Jung WH. Evaluation of Expression of Lipases and Phospholipases of *Malassezia restricta* in Patients with Seborrheic Dermatitis. Ann Dermatol 2013; 25:310-4; PMID:24003273; http://dx.doi.org/10.5021/ad.2013.25.3.310

16. Gácser A, Stehr F, Kröger C, Kredics L, Schäfer W, Nosanchuk JD. Lipase 8 affects the pathogenesis of *Candida albicans*. Infect Immun 2007; 75:4710-8; PMID:17646357; http://dx.doi.org/10.1128/IAI.00372-07

17. Tóth A, Németh T, Csonka K, Horváth P, Vágvölgyi C, Vizler C, Nosanchuk JD, Gácser A. Secreted *Candida parapsilosis* lipase modulates the immune response of primary human macrophages. Virulence 2014; 5:555-62; PMID:24626151; http://dx.doi.org/10.4161/viru.28509

18. Gácser A, Trofa D, Schäfer W, Nosanchuk JD. Targeted gene deletion in *Candida parapsilosis* demonstrates the role of secreted lipase in virulence. J Clin Invest 2007; 117:3049-58; PMID:17853941; http://dx.doi.org/10.1172/JCI32294

19. Silva S, Henriques M, Oliveira R, Azeredo J, Malic S, Hooper SJ, Williams DW. Characterization of *Candida parapsilosis* infection of an in vitro reconstituted human oral epithelium. Eur J Oral Sci 2009; 117:669-75; PMID:20121929; http://dx.doi.org/10.1111/j.1600-0722.2009.00677.x

20. Chow BD, Linden JR, Bliss JM. *Candida parapsilosis* and the neonate: epidemiology, virulence and host defense in a unique patient setting. Expert Rev Anti Infect Ther 2012; 10:935-46; PMID:23030332; http://dx.doi.org/10.1586/eri.12.74

21. Hube B, Stehr F, Bossenz M, Mazur A, Kretschmar M, Schäfer W. Secreted lipases of *Candida albicans*: cloning, characterisation and expression analysis of a new gene family with at least ten members. Arch Microbiol 2000; 174:362-74; PMID:11131027; http://dx.doi.org/10.1007/s002030000218

22. Nagy I, Filkor K, Németh T, Hamari Z, Vágvölgyi C, Gácser A. In vitro interactions of *Candida parapsilosis* wild type and lipase deficient mutants with human monocyte derived dendritic cells. BMC Microbiol 2011; 11:122; PMID:21619700; http://dx.doi.org/10.1186/1471-2180-11-122

23. Asmundsdóttir LR, Erlendsdóttir H, Agnarsson BA, Gottfredsson M. The importance of strain variation in virulence of *Candida dubliniensis* and *Candida albicans*: results of a blinded histopathological study of invasive candidiasis. Clin Microbiol Infect 2009; 15:576-85; PMID:19604278; http://dx.doi.org/10.1111/j.1469-0691.2009.02840.x

24. Pulcrano G, Roscetto E, Iula VD, Panellis D, Rossano F, Catania MR. MALDI-TOF mass spectrometry and microsatellite markers to evaluate *Candida parapsilosis* transmission in neonatal intensive care units. Eur J Clin Microbiol Infect Dis 2012; 31:2919-28; PMID:22644055; http://dx.doi.org/10.1007/s10096-012-1642-6