You are what you secrete: extracellular proteins and virulence in *Cryptococcus*

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Fungal organisms secrete a wide range of biomolecules, including degradative enzymes that are essential for nutrition, toxins, effectors and secondary compounds that modulate interactions with host animals and plants, and a variety of signaling and stress-related proteins\(^1\). As these are likely to be key determinants of virulence and may also be useful diagnostic and therapeutic targets, we investigated the secretome of different strains of the fungal pathogen *Cryptococcus*. Virulent strains secreted predominantly hydrolytic and proteolytic enzymes, while the least virulent strain secreted a range of additional non-degradative proteins including many that lacked secretion signals, some that appear to be ‘moonlighting’, and a number that are known to be allergenic. It appears that in *Cryptococcus*, the secretome may influence virulence both through the presence of harmful enzymes and through the absence of proteins that alert the host defence mechanisms.

*Cryptococcus* is an encapsulated yeast with two predominant pathogenic species: *Cryptococcus neoformans* and *Cryptococcus gattii*. These cause cryptococcosis in animals and humans, with disease ranging from asymptomatic to severe, fatal meningitis. There are a number of differences between *C. gattii* and *C. neoformans* including their preferred environmental niche, basidiospore morphology, drug susceptibility, epidemiology, the clinical manifestations of associated disease, and host susceptibility\(^5\). In addition, there are significant differences among strains within each species. In *C. gattii*, a hypervirulent sub-genotype designated VGIIa has caused a recent significant outbreak of cryptococcosis on Vancouver Island in British Columbia, Canada and in the Pacific Northwest of the United States. In contrast, a closely related sub-genotype designated VGIIb is globally distributed and hypovirulent\(^3\). These differences between *Cryptococcus* species and sub-genotypes provide an opportunity for understanding pathogenicity and disease progression by what are otherwise very genetically similar fungal organisms.

Our laboratory has been using ‘omics approaches to understand virulence in *Cryptococcus*, and used proteomic analysis to characterize the secretome produced by three *Cryptococcus* strains. Two strains were of high virulence (*C. neoformans* and *C. gattii* sub-genotype VGIIa) and the third of low virulence (*C. gattii* sub-genotype VGIIb). In our previous work on *Cryptococcus* proteomics, we found conditions optimized to simulate those encountered in the host induced the production of large amounts of shed capsular material, which interfered with the isolation and identification of proteins\(^4\). Therefore, we developed a novel method of protein capture using BioRad ProteoMiner™ beads, followed by mass spectroscopy. Sixty-seven cryptococcal proteins were identified and only one was common to all three strains. The secretomes of the high virulence *C. neoformans* and *C. gattii* VGIIa strains were similar and mostly consisted of a hydrolytic and proteolytic proteins. In contrast the lower virulence *C. gattii* VGIIb strain had a larger...
number of proteins with a greater diversity of functions (Figure 1). A significant proportion of these proteins are known to have roles in metabolism, signaling/transport, glycolysis and redox processes, and are considered to be canonical intracellular proteins. Published studies have reported very similar proteins in the extracellular milieu of various cell types from other organisms, and there is growing evidence that these may have ‘moonlighting’ functions, where they participate in completely different processes in alternative environments. An additional subset of proteins found only in the C. gattii VGIIb secretome were orthologous to proteins known to elicit an immune response in the host, including the glycolytic proteins enolase and glyceraldehyde-3-phosphate dehydrogenase. Most of these unusual secreted proteins lack secretion signals and are likely to be exported via alternative secretion pathways such as inside microvesicles, which have previously been isolated from Cryptococcus; indeed the regulatory 14-3-3 protein, which is a surface antigen, and is an adventitious Candida albicans cell wall protein. Published studies have reported very similar proteins in the extracellular milieu and are considered to be canonical intracellular proteins. Published analysis of a range of organisms such as bacteria, yeast, mammalian infection is likely to be accidental. Cryptococcus is an environmental fungus, and as it cannot be spread from host to host, mammalian infection is likely to be accidental. Comparative genomic studies have identified genes that are particular to the high virulence strains but their role in virulence is yet to be verified. As secreted biomolecules are mediators of contact between the host and the pathogen, differences in these are likely to influence whether a pathogen will be rapidly recognized and eliminated, or will be able to bypass the host response and use host resources to establish an active infection. The results of our secretome analysis suggest that virulence in Cryptococcus may in part be determined by restricted secretion of proteins likely to elicit an immune response, and that in the absence of these the production and secretion of degradative enzymes enables host invasion.

**References**


**Biographies**

**Leona Campbell** has spent the past 13 years being fascinated by the fungal pathogen Cryptococcus, both as a PhD student and Postdoctoral researcher, primarily in Dee Carter’s lab. Her major area of interest is investigating host-pathogen interactions using ‘omics’ approaches. Leona also enjoys her teaching role overseeing the running of intermediate undergraduate Microbiology practical courses at the University of Sydney. She loves having the opportunity to inspire, and be inspired by, our next generation of microbiologists.

**Dr Matt Padula** is a Lecturer in the School of Biological Sciences and Professional Officer in the Proteomics Core Facility at the University of Technology Sydney. His research lies in the proteomic analysis of a range of organisms such as bacteria, yeast, mammalian

**Figure 1. The secretome of high and low virulence strains of Cryptococcus.** Red triangles: hydrolytic and proteolytic enzymes; Green circles: proteins involved in metabolism, signaling/transport, redox, stress responses or with unknown function.
tissue and cells, plant tissue, parasites, paralysis ticks, coral, snake venom and the pathogenic fungus *Cryptococcus.*

**Liz Harry** is a Professor of Biology and Deputy Director of the ithree institute (infection, immunology and innovation) at the University of Technology, Sydney (UTS). Liz obtained her PhD at the University of Sydney, was an NIH Fellow at Harvard, an Australian Research Council (ARC) Postdoctoral Fellow and an ARC QEII Fellow in the School of Molecular Biosciences at the University of Sydney. She has won an Australian Eureka Prize for Scientific research, and an ASM Frank Fenner Award. Her research focuses on bacterial cell division and antibacterials.

**Dee Carter** is an Associate Professor and head of the Discipline of Microbiology in the School of Molecular Bioscience, The University of Sydney, where she teaches mycology, medical microbiology and molecular biology. Her current research interests focus on using ‘omics approaches to understand fungal pathogenesis and to develop novel antifungal agents. She loves fungi because they are so adaptable and clever, making them excellent pets but also devastating enemies. She is particularly fond of *Saccharomyces* because it fits into the former category, *Cryptococcus* because it fits into the latter, and *Aspergillus* because it manages to straddle both.

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**Morphogenesis and pathogenesis: control of cell identity in a dimorphic pathogen**

Fungal pathogens span all major phylogenetic groupings within the fungal kingdom, infecting animals, plants and other fungi. Intrinsic to their ability to infect a host and survive host defense mechanisms is the capacity to produce the appropriate cell type. The link between morphogenesis and pathogenesis is clear for a number of pathogenic fungi that undergo a phase transition known as dimorphism (or dimorphic switching)\(^1\). Dimorphic fungi are able to alternate between multicellular filamentous growth, characterised by highly polarised hyphal growth, and unicellular growth with yeast cells dividing by budding or fission. This trait is strongly linked with virulence in the important human pathogens *Blastomyces dermatitidis*, *Candida albicans*, *Coccidioides immitis*/*posadasii*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*/*lutzii*, *Talaromyces marneffei* (formerly named *Penicillium marneffei*) and *Sporothrix schenckii*\(^4\). Uncovering the mechanisms that control morphogenesis during dimorphic switching and the physiological properties of the hyphal and yeast cell types is crucial to understanding pathogenicity.

Prevalent in South-East Asia and the surrounding regions, *T. marneffei* causes a deadly systemic infection in immunocompromised hosts\(^2,3\). The rapid rise in *T. marneffei* infections associated with the worldwide HIV pandemic led to it being described as an AIDS-defining pathogen\(^1\). While there are sporadic reports of *T. marneffei* infections in ‘immunocompetent hosts’ the immune status has not been adequately tested in these cases, and the term ‘immunocompetent’ is often used interchangeably (and incorrectly) in these reports with HIV negative status. The ecological niche of *T. marneffei* is unclear, but there is a strong association with a number of bamboo rat species in endemic areas\(^3,4\). *T. marneffei* is unique as the only member of the very large Eurotiales order that can undergo a dimorphic switch, and the only *Penicillium* species within this order known to be a pathogen\(^3,6\).