8.3d Characterization of glycosphingolipid/fatty-acid-linked aspartyl proteases in Candida glabrata role in pathogenicity

Rajinder Kaur
Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India

8.3.3 How to Fungal Cell Wall Glycan Can Modulate the Immune Response?, September 23, 2022, 3:00 PM - 4:10 PM

Candida glabrata is the second most frequent yeast pathogen found in Candida bloodstream infections, depending upon the geographical location. C. glabrata, which belongs to the non-albicans clade, possesses a distinct set of virulence attributes which make it either a less-potent or predilection in macrophages, adheres to host and eukaryotic surfaces and survive a wide range of stresses. Our research is focusing on unraveling the strategies that C. glabrata employs to survive the rampant, hostile host-environment and evade host immune responses. Toward this end, we are delineating the cellular processes, that are primarily involved in the pathogenesis of C. glabrata. We have isolated and characterized C. glabrata genes that encode for two, fungal glycosylphosphatidylinositol linked (CgPgps1) and fatty-acid linked proteases (CgPgps2). We have recently characterized the secretion of C. glabrata wild-type and aspartyl protease-deficient mutant strains and found that the secretion of the secreted aspartyl proteases is both beneficial outcomes and key modulators of the C. glabrata secretion. Further, elucidating the role of CgPgps1 in the suppression of the host pro-inflammatory immune response, we have identified the C. glabrata-dependent protein (CgPgps1) for characterization in the previous studies. The combination of CgPgps1-mediated immunity and the pathophysiological role of C. glabrata will be presented.

8.4b Population biology of fungal fungus Thopzyophycon e incael

Vit Hubka 1
1Charite University, Faculty of Science, Prague, Czech Republic

8.4.4 Causes of animal mycoses, September 23, 2022, 3:00 PM - 4:30 PM

Thopzyophycon e incael is a main cause of dematitism in hedgehogs and is increasingly reported from human infections worldwide. It is found in wild European hedgehogs (Erinaceus europaeus) but also in the African four-toed hedgehogs (Atelerix albigena) in increasing numbers per animal worldwide. Little is known about the reservoir and population genetics of this pathogen despite its increasing importance in clinical practice. Notably, whether there are different populations or even cryptic species associated with different hosts or geographic regions is not known. To address these questions, we collected 161 isolates, per- formed phylogenetic, ecological, and population analyses, determined virulence traits, and characterized morphology and phenotypic traits. Multiple phylogenetic and microsatellite analysis supported T. e incael as a monophyletic species, in contrast to highly incon- gruent results from population analyses. Therefore, one species mainly to students and the second to Erinaceus haga, were identified inside T. e incael, and slight differences in the size of macromorph and azoteolytic susceptibility were observed among them. Although the process of speciation into two lineages is ongoing in T. e incael, there is still gene flow between these phenotypically distinct lineages, with notable intraspecies variation in genotype and phenotype. The data from wild hedgehogs indicated that usual reproduction in T. e incael and the de novo infection of hedgehogs from sea are probably case events and that clonal horizontal spread is strong resistant. The molecular typing approach used in this study is applicable to facultative and obligate phytopathogenic T. e incael species. Our findings demonstrate that T. e incael is a pathogenic fungus in hedgehogs, to wild animals and humans. The results of this study also highlighted the need to use a mycological phylotype ideally in combination with other circulating molecular markers to understand the species boundaries of dematitoplasms.

8.5c MLST genotyping and phylogenetics of AD-hybrids

Massimo Cigolli, 1 Minh Chen, 1 Jinqiu Xu, 1 Moen Hsueh-Ching, 2 Dong-Hsun Yang, 3 Volker Roberts, 2 Marie Deonnie Oliveira, 4 Joan-Incivis Silva, 5 Waldemar Meyers, 6,7 Magdalena Flosa, 8 Urszula Neesan, 8 Patricia Escardino, 9 Andrea Pumpl, 9 Frederik Rogier, 10 Sebastien Bertolino, 10

1University degli Studi di Milano, Italy
2Changhong Hospital, Shanghai, China
3McMaster University, Hamilton, Canada
4NHM, Berlin, Germany
5Robert Koch Institute, Berlin, Germany
6Institut Pasteur, Paris, France
7University of Brighton, Brighton, UK
8Sydney University, Sydney, Australia
9Curtin University, Perth, Australia
10University of Würzburg, Würzburg, Poland

8.5.5 Geotyping of Cryptococcus neoformans and C. gattii, September 25, 2022, 3:00 PM - 4:30 PM

Objectives: In a previous study a set of new molecular-type-specific primers were designed to apply the standard DHSM consumes multiple-locus sequence typing (MLST) scheme to Cryptococcus neoformans AD hybrids. In the present study, we report the preliminary results of the investigations by MLST of a large number of AD hybrids with the aim to characterize the following, genotypes, and population genetics.

Methods: A total of 50 AD hybrids isolated from different parts of the world and from different sources were genotyped by MLST. Minimum spanning tree and group analysis were generated by comparing hybrid genotypes and by constructing separately either allele-A and allele-D portions of the hybrid genotypes to the haplotypes recorded in the MLST global database. Results: Analysis identified 32 hybrid genotypes grouped in distinct asmatous clusters (C22, C41, and C42) including 12 isolates each. Both C22 and C42 clusters included isolates from different countries and continents but the former group had only isolates with mating type AD and the latter those with both mating type A and D. Clusters C41 included only isolates from China. Heterogeneous allele combinations in each of the MLST profiles but presented two or three combinations more frequent than the others. In some isolates, one or more alleles were not amplified and multiple genotypes, and therefore, the number of genotypes assessed was considerably lower. A total of 22 MLST profiles were identified by analyzing separately the allele-A combinations of the hybrids. Comparison with all MLST profiles of Y17, Y18, and Y19 included in the MLST global database showed that the allele-A portion of the hybrid genotypes was grouped in two Y17 and Y18 or Y19 clusters. None of the investigated hybrids showed genotypic differences from Y17 and Y18 genotypes. Similarly, when the MLST profiles of allele-D portion of hybrids was compared to all Y17 genotypes present in the global MLST database, few clusters were identified but, in this case, mostly originated from one strain for each allele-D genotype.

Conclusions: These preliminary results suggest that the AD hybrids have inherited origins from the mating of A haplotype very common in both clinical and environmental isolates and D haplotypes that are not circulating or present in very rare cases. Therefore, it is likely that the hybrids originated in the environment where Y17 genotypes diversity is higher and sample AD combinations can occur. Sequencing of further AD hybrids is in progress to confirm these results.

8.5d Cryptococcus neoformans and Cryptococcus gattii clinical isolates from Colombia develop heteroresistance to fluconazole at high concentrations

Javier Meijerend, 1 Silvia Cearval-Valenzuela, 2 Patricia Escardino, 9 Carmela Farinacci

1Estudios en Translacional y Materno Neonatal Microbiología, Microbiología, School of Medicine and Health Sciences, Universidad Del Rosario, Bogota, Colombia
2Group of Microbiology, National Institute of Health, Bogota, Colombia

8.5.5 Geotyping of Cryptococcus neoformans and C. gattii, September 25, 2022, 3:00 PM - 4:30 PM

Introduction: Cryptococcus is a worldwide mycoses caused by Cryptococcus neoformans and Cryptococcus gattii. Although resistance to antifungals is infrequent, isolates with decreased susceptibility to fluconazole have been reported globally, including Colombia, which may be due to: 1) heteroresistance, defined as the ability to adapt to increasing concentrations of this antifungal, and 2) point mutations in the ERG11 gene encoding the fluconazole target enzyme, lanosterol 14α-demethylase. Objective: To determine the development of heteroresistance to fluconazole in C. neoformans and C. gattii clinical isolates from Colombia and to evaluate and quantify the ERG11 gene of the isolates to seek resistance that might characterize resistant or heteroresistant phenotypes.

Methods: The minimum inhibitory concentration (MIC) to fluconazole was determined in 18 and 24 isolates of C. neoformans and C. gattii, respectively, using broth microdilution. Heteroresistance was evidenced by evaluating each isolates on YPD agar that contained fluconazole at concentrations equal to the MIC of each isolate. Heteroresistant colonies were then regrown in initial concentrations of fluconazole.

Results: All isolates were susceptible to fluconazole with MICs of 1 μg/mL (n = 2), 2 μg/mL (n = 6), 4 μg/mL (n = 17), 8 μg/mL (n = 25), 16 μg/mL (n = 2), and 32 μg/mL (n = 1). However, all isolates developed heteroresistant colonies, with increased MICs in the range of 2 to 16 μg/mL. MICs for 9 (5.5%) of C. neoformans and 8 (3.3%) of C. gattii, grew up to 64 μg/mL of fluconazole, which is the MIC that define resistance to this drug, and 1 (1.2%) isolate of C. neoformans and 4 (1.7%) of C. gattii, grew up to 128 μg/mL of fluconazole, which is the MIC that define the process of heteroresistance. In conclusion, clinical isolates of C. neoformans and C. gattii that develop heteroresistance to fluconazole in high concentrations in Colombia, which is important since this characteristic contributes to the epidemic of cryptococcosis during therapy with this triazole.
 discovery (Yi et al., 2021). We here screened ~2,000 crude extracts from mycobacteria in an in vitro C. albicans-mammalian epithelial cell infection model. Typically, when oral epithelial cells (BECs) are infected by C. albicans, the fungus proliferates, forms hyphae, and invades and damages the monolayer. In our assay, we estimate the damage to epithelial cells by released lactate dehydrogenase. We also note changes to the growth and morphology of the fungus. Based on these readouts, we assign antivirulence and antifungal ranks to each extract, and confirm top-ranked hits with an independent prophylactic single-cell based assay for host cell damage, and a cytokinetic assay of fungal metabolic activity. We found that several of the top-ranked antifungal extracts also showed effects on a multi-drug resistant strain of C. auris.

Using an established pipeline, we identified several of the antifungal and antivirulent bioactive components in these extracts. After scaling the production of promising lead compounds, we will test their durability on clinical Candida spp. strains, and identify their mode of action using large-scale Candida spp. knock-out libraries and multi-omics approaches.

Our tested extracts are likely to contain new classes of non-toxic antifungals that can potentially treat infections by multidrug-resistant fungi. We identified and confirmed several mycobacteria extracts that protected mammalian epithelial cells without severely affecting the fungus’ growth, which are, therefore, considered antivirulent.

**S9.4a** Oral infections by melanized fungi Curvularia lunata and Lasiodiplodia theobromae: Antifungal susceptibility and clinical outcome

Sanchita Mitra, Prashant Garg, Somanathula Murthy, Vivek Pravin Dave
LV Prasad Eye Institute, Hyderabad, Hyderabad, India

**Purpose:** To report antifungal susceptibility and clinical outcomes in melanized fungal isolates of Curvularia lunata and Lasiodiplodia theobromae from ocular infections.

**Methods:** Antifungal susceptibility testing was performed by broth microdilution testing, following Clinical and Laboratory Standard Institute guidelines, of 17 C. lunata and 11 L. theobromae isolates from mucocutaneous infections of microbial keratitis or fungal endophthalmitis patients. Isolates resistant to ≥2 classes of antifungals were considered as multidrug-resistant (MDR). The panel of antifungals tested were amphotericin B, natamycin, voriconazole, fluconazole, itraconazole, posaconazole, and caspofungin.

Results: Voriconazole showed the highest susceptibility (≤83.3% isolates) followed by natamycin (80%), fluconazole (80%), itraconazole (76.7%), posaconazole (76%), and caspofungin (44.7% each) and lastly amphotericin B (63.3%). For treatment, all patients received topical natamycin, and few received additional oral ketoconazole or intravitreal voriconazole. MDR isolates led to the poorest clinical outcomes (P=0.015) in patients. But natamycin resistance alone did not show unfavorable outcomes (P=0.28), though this was the most frequent drug used topically in fungal ocular infections.

**Conclusions:** Melanin-coated fungi causing ocular infections have varying susceptibility to different antifungal agents. Most effective drug as seen in vitro in our study was voriconazole. Significant resistance to amphotericin B, which is the most common antifungal used in intravitreal injections, was noted. MDR isolates overall had poorer clinical outcomes.