SS.3d Characterization of glycosylphosphatidylinositol-linked aspartyl proteases in Candida glabrata Role in pathogenicity

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SS.3 Hone the Pangal Cell Wall Glycan Can Modulate the Immune Response*, September 23, 2022, 3:00 PM - 4:10 PM

Candida glabrata is the second most frequent cause of pathogen found in Candida bloodstream infections, depending upon the geographical location. C. glabrata, which belongs to the Nakasonea clade, possess a distinct set of virulence attributes which are critical for its ability to persist and proliferate in macrophages, adhere to host, and epithelial surfaces and survive a wide range of stresses. Our research is focusing on unraveling the strategies that C. glabrata employs to survive the nutrient-poor hostile environment and evade host immune response. Toward this end, we are delineating the cellular processes that are involved in the virulence attributes of C. glabrata. This talk will be highlighting the role of C. glabrata in the suppression of the host pro-inflammatory immune response, we have identified the fucoido-like protein CgPep2 as a substrate of the CgPep1 protease and demonstrated that the proteolytic activity of CgPep1 is pivotal in evading the immune response and function of CgPep1 in pathogenesis. These findings underpinning the importance of multifunctional CgPep1 in the physiology and pathogenesis of C. glabrata will be presented.

SS.4b Population biology of hedgehog fungus Thyrachyptorn e ineradicabilis

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SS.4a Causes of animal mycoses, September 23, 2022, 3:00 PM - 4:10 PM

Thyrachyptorn e ineradicabilis is a main cause of dermatomycoses 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 hedgehog (Atelerix albiventris), a popular pet animal worldwide. Little is known about the taxonomy and population genetics of this pathogen despite its increasing importance in clinical practice. Notably, whether there are different populations or even species associated with different hosts or geographic regions is not known. To answer these questions, we collected 165 isolates, performed multilocus sequence analysis, population genetic analyses, determined mating type, and characterized morphology and physiology. Multiple phylogene and microsclerotium analysis supported T. ineradicabilis as a monophyletic species, in contrast to highly incongruent results in population genetic analyses, one species mainly to hedgehogs and the second to European hedgehogs, were identified inside T. ineradicabilis, and slight differences in the size of microsclerotia and antifungal susceptibility were observed among them. Although the process of speciation into two lineages is ongoing T. ineradicabilis, there is still gene flow between these populations, indicating that hedgehogs play a role in the transmission of the fungus. The data from wild hedgehogs indicated that usual reproduction in T. ineradicabilis and the mode of infection from hedgehogs to wild animal cases and that clonal horizontal spread is strong. The molecular typing approach used in this study provides a framework for further phylogenetic, morphological, and molecular characterization of hedgehogs.

The results of this study also highlighted the need to use a mycology philosophy ideally in combination with other clinical molecular markers to understand the species boundaries of dermatomycoses.

SS.5c MLST genotyping and phylogenetics of AD-hybrids

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SS.5 Genealogy of Cryptococcus neoformans and C. gattii, September 23, 2022, 3:00 PM - 4:10 PM

Objectives: In a previous study a set of new molecular-type specific primers were designed to apply the standard ISHAM consensus multilocus 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 identifying the circulating genotypes, their phylogenetics, and population genetics.

Methods: A total of 50 AD hybrid isolates from different parts of the world and from different sources were genotyped by MLST. Minimum spanning tree (MST) analysis with the resulting sequences were generated by computer hybrid genotypes and by comparing separately either allele A and allele B of the hybrid genotypes to the haplotypes recorded in the MLST global database. Results: Analysis identified 32 hybrid genotypes grouped in these new strains mainly CC12, CC21, and CC31 including 12 isolates each. Both CC12 and CC21 clustes included isolates from different countries and continents but the former group only with mating type A and B whereas the latter those with mating type allele A/As. Clusters CC12 included only from Egypt. However, alleles A and B combinations in one of the strains has previously two or three combinations more frequent than the others. In some isolates, some A allele were not amplified after multiple attempts, and therefore, further analysis was not possible. A total of 21 MLST profiles were identified by analyzing separately the allele A and B combinations of the hybrids. Comparison with all MLST profiles of YNN, YNB and NNB included in the MLST global database that showed allele A portion of the hybrid genotypes was grouped in four YNN and YNB clusters. In none of the investigated hybrids, a genotype was identified except from YNB genotypes. Similarly, when the MLST profiles of allele A and B hybrids were compared to all NNB genotypes present in the global MLST database, few clusters were identified but, in this case, mostly originated from strains from the central Europe. Conclusions: These preliminary results suggest that the AD hybrids have evolved independently from the mating A haplotype very common in both clinical and environmental isolates and D haplotype that are not circulating at present or very rare. Therefore, it is a likely that hybrids originated in the environment where YNN genotypes; diversity is higher and satisfied AD combinations can occur. Sequencing of further AD hybrids is in progress to confirm these results.

SS.5d Cryptococcus neoformans and Cryptococcus gattii diseases in Colombia develop heteroresistance to fluconazole at high rates

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SS.5 Genealogy of Cryptococcus neoformans and C. gattii, September 23, 2022, 3:00 PM - 4:10 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 the drug antifungals, and 2) point mutations in the ERG11 gene encoding the fluconazole target enzyme, lanosterol 14-dehydro-lyase.

Objective: To determine the development of heteroresistance to fluconazole in C. neoformans and C. gattii clinical isolates from Colombia and to analyze and sequenced ERG11 gene of the isolates to seek for mutations that might contribute to antifungal resistance or heteroresistance phenotypes.

Methods: The minimum inhibitory concentration (MIC) to fluconazole was determined in 28 and 24 isolates of C. neoformans and C. gattii, respectively, using broth microdilution. Heteroresistance was evidenced by plating each isolate on YPD agar that contained fluconazole at concentrations equal to the MIC of each isolate. Heteroresistant clones were then replaced in repeated passages of fluconazole-containing media.

Results: All isolates were susceptible to fluconazole with MICs of 1 µg/mL (n = 2), 2 µg/mL (n = 4), 4 µg/mL (n = 17), 8 µg/mL (n = 23), 16 µg/mL (n = 5), and 32 µg/mL (n = 1). However, all isolates developed heteroresistance clones, with increasing concentrations of fluconazole from 2 µg/mL (n = 8) to 4 µg/mL (n = 5) to 8 µg/mL (n = 3) of C. gattii, grown up to 64 µg/mL of fluconazole, which is the MIC that defines resistance to this drug, and 1 (1.2%) isolates of C. neoformans and 4 (16.7%) isolates of C. gattii were found up to 2 µg/mL of fluconazole, which is the MIC that defines resistance to this drug.

Conclusions: 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 failure of cryptococcal diagnosis treatment with this triazole.