P323
Virulence not required? Albumin promotes pathogenicity of (non)-damaging *Candida* strains

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

The pathogenicity of the dimorphic yeast *Candida albicans* is associated with filamentation, adhesion, invasion, and production of the toxin Candidalysin. However, there are certain clinical isolates and other *Candida* spp., that cause infection independent of filamentation or the production of Candidalysin. Consequently, these strains and species are often non-damaging *in vitro*, this does not correlate with their potential to cause infection in patients. We hypothesize that specific host factors, which trigger pathogenicity, are absent in *in vitro* models, and thereby not reflecting the situation in the host.

To determine the impact of albumin, the most abundant protein in the human body, vaginal epithelial cells were infected with different *C. albicans* strains and *Candida* species. Interestingly, after prolonged infection (45 h) albumin increased the damage potential, even in otherwise non-damaging and non-filamentous strains. This included deletion mutants deficient in filamentation, als3 adhesin/invasin, thigmotropism, or Candidalysin production. Yet, the increased damage was likely not solely an effect of increased growth and nutrient competition between the fungus and epithelial cells. Reduced damage in presence of protease inhibitors and albumin hint toward the role of proteases in the utilization of albumin. Albumin enhanced *C. albicans* metabolism, by stimulating the utilization of various nitrogen sources. This metabolic adaption could explain the advantage and enhanced growth as a strain and species-independent feature.

Our data suggest that common host factors can impact *C. albicans* to cause damage independent of adhesion, invasion, filamentation, and toxin production. Possibly, also other host-derived factors can drive the pathogenic potential of fungi through unresolved mechanisms.

P324
Phylogenetic and ecological overview of Onygenales

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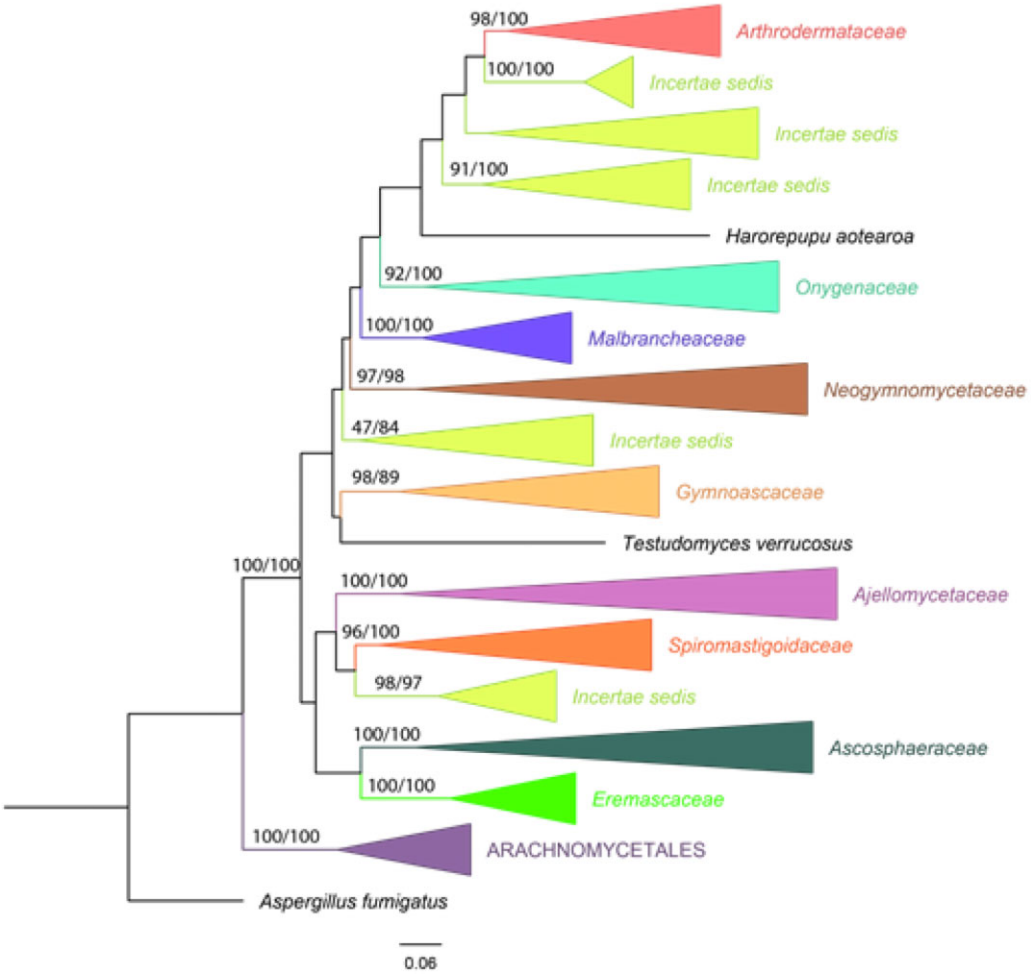
Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

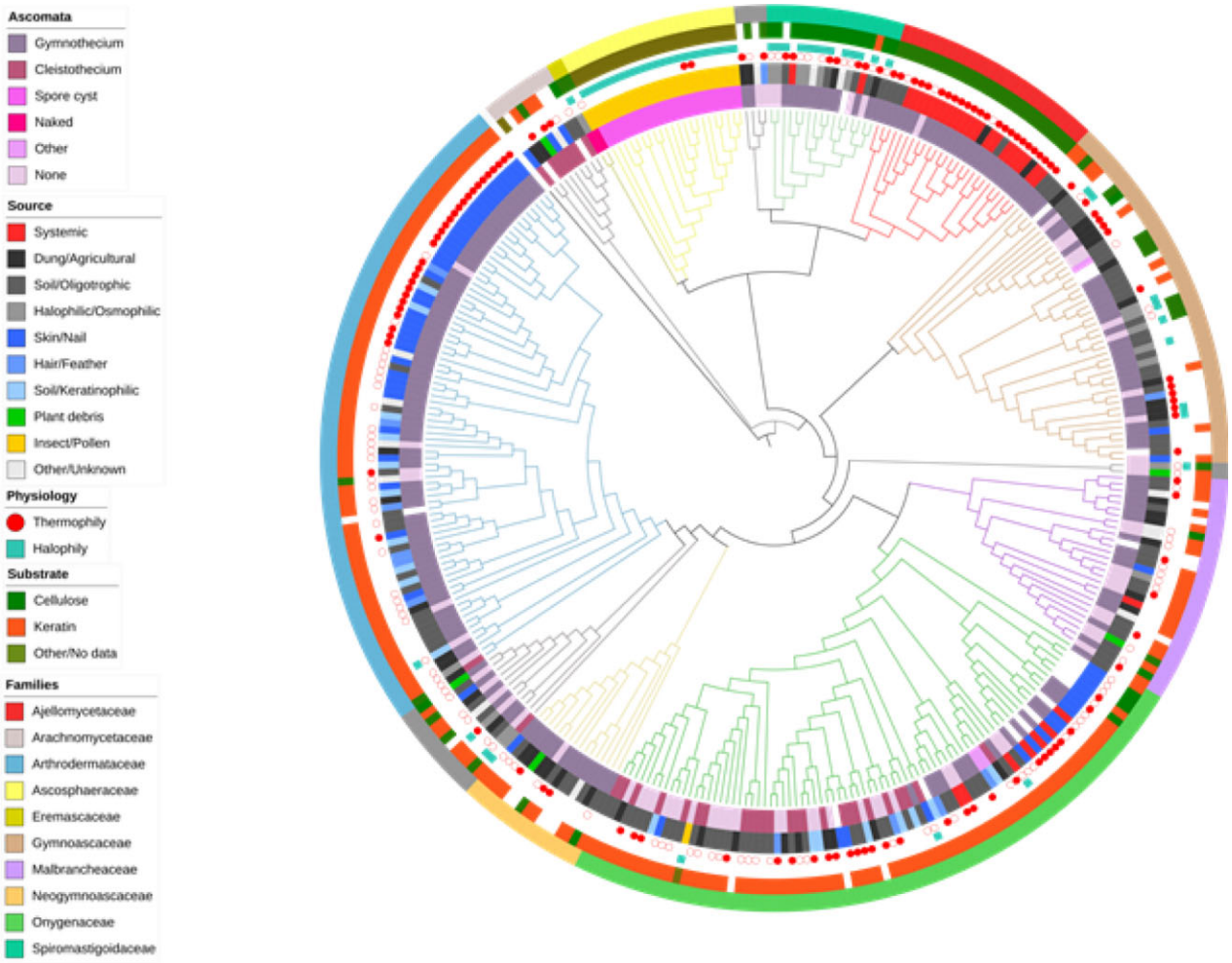
Objectives: To evaluate the general taxonomy and phylogeny of the order Onygenales using ecological, morphological, and molecular data, stimulate awareness of correct identification of neglected groups in the order, and contribute to the stabilization of the nomenclature.

Methods: In total 97 genera, 385 species, and 553 strains were analyzed in this study. The ITS, LSU, TUB, TEF3, and RP60S gene regions were amplified and sequenced. Sequences for the RPB1, RPB2, and TEF1 regions were retrieved from the NCBI nucleotide database. Whole genome data for 53 strains were also included in phylogenetic tree analyses. Ecology and ascomata morphology for the type species were retrieved from the literature. Phylogenetic trees were constructed using the maximum likelihood methods implemented in IQ-TREE software and MRBAYES v3.2.7 on the CIPRES portal. Additionally, relative divergence time within Onygenales was estimated based on the RelTime method implemented in MEGA 7.

Results: A total of 1667 sequences for LSU ($n = 421$), ITS ($n = 519$), TUB ($n = 189$), RP60S ($n = 123$), TEF1 ($n = 119$), TEF3 ($n = 144$), RPB1 ($n = 71$), and RPB2 ($n = 97$) were examined. The results of the combined data analysis yielded 14 clades with ≥90% support for Bayesian probability and ≥80% support for maximum likelihood analyses. Families, based on their type genera and type species, were resolved as *Ajellomycetaceae, Arthrodermataceae, Ascosphaeraceae, Eremascaceae, Gymnoascaceae, Onygenaceae,* and *Spiromastigoidaceae* (Fig. 1). Two families were newly introduced as *Malbrancheaceae* and *Neogymnomycetaceae*. The family *Nannizziopsidaceae* clustered amidst members of *Onygenaceae*. The ecological preferences were classified as soil/oligotrophic, soil/keratinophilic, dung/agricultural, skin/nail, hair/feather, insect/pollen, osmotic habitats, systemic, plant, and other/unknown (Fig. 2). Almost all families in the order have members that can be found on skin and nails, which can cause asymptomatic or symptomatic infections, or members that are able to grow at 37°C and cause systemic infections. Four main types of ascomata morphology were noted: cleistothecium, gymnothecium, spore cyst, and naked fruitbody. The results of RelTime analysis showed that the diversification of species in Onygenales occurred at 103 Mya. The earliest species of the order were found in *Gymnoascaceae*, while the most recent species were found close to *Arthrodermataceae*.

Conclusion: Determination of the borderlines in the order can be difficult because of the effects of chosen methods, number of samples, number of genes, and also the choice of outgroups. Taxon sampling and inclusion of both type species and related genera in analyses are particularly essential to minimize changes and stabilize nomenclature for longer periods. Providing molecular data for the isolates and making them publicly available is also important to prevent taxonomic disagreements. Significant ecological traits that determine evolution in Onygenales are osmophily, thermophily, cellulolysis, eutrophism, oligotrophism, keratinolysis, and thermal dimorphism. Morphological and physiological characteristics may be informative for habitat choice and evolutionary processes. Cellulolytic and osmophilic abilities might be ancestral characteristics in Onygenales. Even though most of the species are found in soil and are non-pathogenic, environmental and host alterations can lead to the emergence of new fungal pathogens among soil fungi. Therefore, Onygenales continues to deserve close attention.





P325

Trend of cryptococcal meningitis in patients attending a teaching hospital in north-east india — a single center study

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: To determine the changing trend of Cryptococcal meningitis in patients attending Regional Institute of Medical Sciences Hospital, Imphal.

Methods: A total of 142 cerebrospinal fluid (CSF) samples collected from patients with suspected meningitis from January 2013 to January 2022 were analyzed in the department of Microbiology, Regional Institute of Medical Sciences, Imphal. The samples were subjected to India ink preparation (IIP), Gram stain, Cryptococcal antigen (CrAg) testing, fungal culture in Sabouraud Dextrose agar (SDA) medium, and Bird Seed Agar.

Results: Out of 142 CSF samples received, direct examination by IIP revealed *Cryptococcus* in 28 cases (19.7%) whereas, capsular polysaccharide antigen testing using lateral flow immunochromatography kit (CRYPTO-PS) detected a total of 40 cases (28.2%). Culture showed growth of fungus in 22 samples and all were from CrAg positive samples. Maximum number of cases were detected in the year 2019 followed by 2018. Immunosuppression (AIDS) was an important underlying factor. Recurrence was seen in three patients and two cases who were on Cycloserine succumbed while undergoing treatment.

Conclusion: Cryptococcosis continues to be a serious fungal infection among AIDS patients in spite of HAART. High index of clinical suspicion and microbiological examination is necessary to improve clinical outcomes. Whenever immunosuppressive drugs are administered in HIV patients treated earlier for cryptococcosis, monitoring the patient for likely recurrence is to be borne in mind to improve the overall clinical outcome. CrAg testing is an additional armamentarium for the diagnosis of cryptococcosis in the absence of culture positivity.

P326

DNA replication initiator proteins stabilize the kinetochore in the human fungal pathogen *Cryptococcus neoformans*

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: DNA replication licensing ensures maintenance of the ploidy state by limiting DNA replication once per cell cycle. The mini-chromosome maintenance (MCM) complex is an evolutionarily conserved DNA helicase in eukaryotes that aids this process. Upon activation in the S phase by several protein kinases, the MCM complex unwinds the double-stranded DNA and moves away from DNA replication origins preventing re-initiation of replication in the remainder of the cell cycle. Deregulation of MCM function can lead to malignant transformation of proliferating cells as indicated by their upregulated expression in human cancer and pre-cancerous cells. MCMs, therefore, serve as important biomarkers and potential targets for anti-cancer drugs. This study aims to decipher the effect of deregulated expression of the MCM complex on the cell cycle events of the human fungal pathogen, *Cryptococcus neoformans*, which is more similar to metazoans than other budding yeast. Aneuploidy-mediated drug resistance is a common mechanism in many major human fungal pathogens including *Cryptococcus* and *Candida*.

Methods: We generated conditional mutants of individual subunits of the MCM complex in *C. neoformans* and assayed the impact of their altered expression on cell cycle events. We also tested the alternative role of MCM subunits in the assembly of the kinetochore, a vital component of the chromosome segregation machinery.

Results: Our screen with MCM conditional mutants identified two *in vivo* functional subcomplexes that comprise the MCM complex in *C. neoformans*. Although upregulated expression of either Mcm2 or Mcm3 does not affect cell cycle progression, overexpression of either Mcm6 or Mcm7 led to the accumulation of cells in the large bud stage with nuclear segregation defects. This work provides evidence for the first time for a mitotic role of pre-replication complex proteins, MCM 2-7 complex in *Cryptococcus*. Depletion of Mcm2 led to arrest of cells in the S and G2/M stages of the cell cycle with defects in nuclear segregation. Localization and expression of several kinetochore proteins upon depletion of Mcm2 established that Mcm2 is vital for kinetochore assembly/integrity. Although the centromeric histone H3 variant, CENP-A, remains largely clustered, the outer kinetochore did not mature indicating that Mcm2 alone or as part of the MCM complex plays a role in kinetochore assembly/integrity and thereby in chromosome segregation.

Conclusion: A conserved eukaryotic DNA helicase, MCM 2-7 complex, has an unexplored non-canonical role in kinetochore assembly/integrity and chromosome segregation.

P327

Prevalence of orofacial Mycoses in COVID-19patients: experience from a tertiary care center in Northern India

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: Due to coronavirus disease (COVID-19) a new group of patients at risk emerged with COVID-19-associated mycoses (CAM). The studies, evaluating the prevalence of CAM are missing in India.

Method: To assess CAM prevalence in a tertiary care hospital in India, we applied direct microscopy, fungal culture, and histopathology on respiratory specimens of 285 critically ill COVID-19 patients admitted between September 2020 and March 2022.

Result: Among the 285 patients, 187 were male, and 98 were female. A total of 34.03% had mucor (33.01% *Rhizopus arrhizus*;1.02% *R. microsporus*), 59.51% had *Aspergillus* (50% *Aspergillus flavus*; 41.17% *A. fumigatus*; 2.94% *A. terreus*; 2.35% *A. niger*), 3.04% had both (*Rhizopus arrhizus* + *Aspergillus flavus*), and 3.01% (*Schizophyllum* sps 5; *Fusarium* 1, *Paecilomyces variotii* 1) had other types of mycosis on fungal smear and culture.

Conclusion: Consistent with others, our findings underline the importance of microbiological/pathological assessment in patients with predispositions for COVID-19-associated mycoses but due to the low prevalence, a routine screening seems not to be indicated currently. However, multicenter studies are desirable for substantiation of findings. A high index of clinical suspicion, diagnosis at an early stage, and use of antifungal agents are essential for a successful outcome.