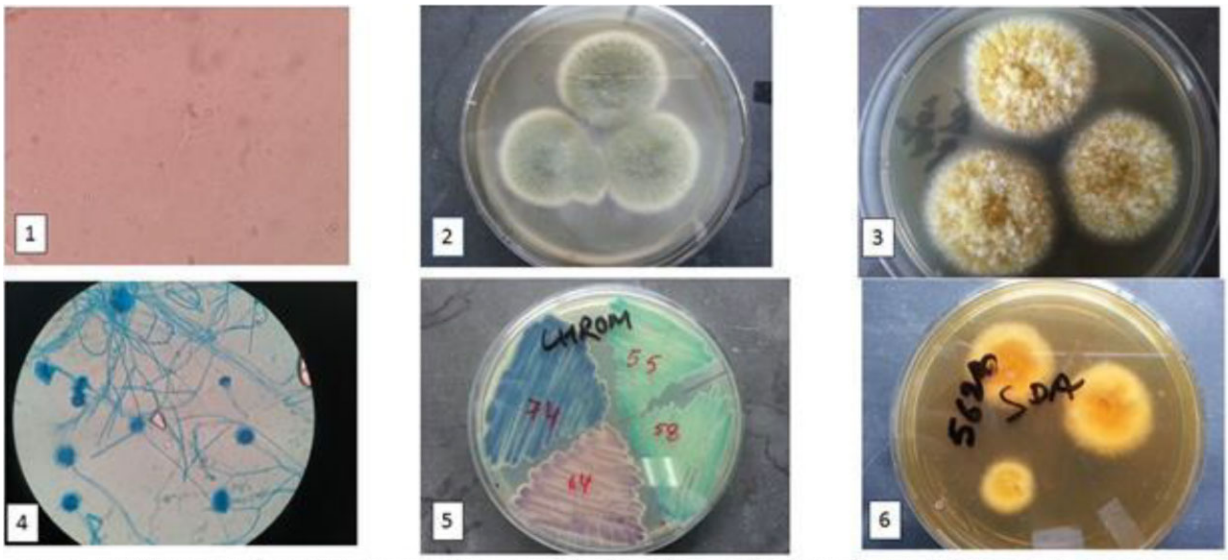

Figure. **1.** KOH mount of ear discharge revealing septate hyphae of *Aspergillus* due to having dichotomous branching when observed under a 40X Olympus CX21 compound microscope. **2.** SDA showing growth of *Aspergillus fumigatus* after 5 days of incubation, **3.** SDA having growth of *Aspergillus flavus*, **4.** *Aspergillus fumigatus* in the LPCB preparation exhibiting conidiophores, vesicles, phialides and conidia, **5.** HiCrome Candida Differential agar disclosing *Candida albicans*-green, *Candida krusei*-pink while *Candida tropicalis* –blue, and **6.** *Acremonium* growth on SDA after 14 days of incubation

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Role of a D-amino acid oxidase (DAO) in an efficient human commensal *Candida albicans*

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

Objectives: *Candida albicans* is an opportunistic fungal pathogen residing as a harmless commensal component of the human microbiota. In the gut, *C. albicans* efficiently colonizes and successfully competes to limiting nutrient resources and overcomes the detrimental effect of an array of secondary metabolites released by the host and the dominant bacterial microflora. Among the metabolites, several free D-amino acids are released into the gut lumen, especially as a result of the degradation of bacterial cell walls. We sought to identify the genes that might confer advantages to *C. albicans* in metabolizing D-amino acids in diverse morphotypes, which might confer a competitive advantage.

Methods: All *C. albicans* genetically modified strains were constructed using a standard lithium acetate transformation method. The D-amino acid utilization and growth of *C. albicans* strains were assessed by spot dilution assays on minimal media agar plates at 30°C. Morphological switching between the yeast and hyphal forms was assessed in liquid media using hyphal-inducing conditions in the presence or absence of D-amino acids. Biofilm assays were performed using YNB-galactose media (with or without D-amino acids) on serum-treated 6-well plates at 30°C.

Results: *C. albicans* genome harbors three ORFs putatively encoding D-amino acid oxidase genes- DAO1orf19.3065, DAO2orf3365, and IFG3orf19.944. D-amino oxidase genes are lost from the genomes of several fungal species belonging to the Saccharomycetaceae family, including the free-living Saccharomyces cerevisiae. We found that *C. albicans* utilizes and grows on D-leucine, an amino acid known to be released by the gut bacteria into the intestinal lumen. This utilization is dependent on the growth morphotypes since the cells growing in biofilm fail to metabolize D-leucine. However, deletion of IFG3 but neither DAO1 nor DAO2 completely abrogates D-leucine utilization capabilities of *C. albicans*. The null ifg3 strain is moderately growth-sensitive in presence of D-alanine, an amino acid abundantly present and synthesized in the gut by bacteria for incorporation into peptidoglycan in their cell walls. By comparing Ifg3 protein levels in a leucine auxotrophic strain in different growth modes, we found that the expression of Ifg3 protein increases several folds in the biofilm growth mode in presence of D-leucine, but cells fail to grow optimally and make three-dimensional biofilm. Furthermore, ectopic expression of CaIFG3 in S. cerevisiae, which lost all D-amino oxidase genes, helps the organism to utilize D-leucine as a nutrient resource as well as overcome its inhibitory effect on growth.

Conclusion: Our results indicate that Ifg3 provides *C. albicans* the ability to metabolize D-leucine and helps it to overcome the growth inhibitory effect of D-alanine. Based on these primary observations we speculate a crucial role of Ifg3 in providing a competitive advantage against the resident microflora in the gut where continuous turnover of D-amino acids is crucial in maintaining host-microbe symbiotic interactions. Further *in vivo* commensalism experiments in mice will provide important clues on the role of this enzyme in *C. albicans*'s ability in gut colonization.

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A screen to identify the regulators of genome stability in the human commensal *Candida albicans*

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Objectives: Cell division is a well-regulated process ensuring high fidelity propagation of genetic material to maintain genome stability. A plethora of proteins in distinct cellular pathways, like DNA replication, repair, and segregation contribute to a stable genome. Defects in either of these processes are sensed by cellular surveillance mechanisms ensuring faithful segregation of duplicated DNA during cell division. Failure to correct these defects leads to aneuploidy and rearrangements which may affect the cell viability. On the other hand, rearrangements in the genome are a well-known mechanism for attaining drug resistance in fungal pathogens including the human commensal *Candida albicans*. With a major percentage of the genome being uncharacterized in *C. albicans*, the regulators of genome stability are poorly studied. To gain a better understanding of the regulation of genome stability and antifungal resistance, we aimed to identify and characterize novel genome stability regulators in *C. albicans* using an overexpression ORFeome.

Methods: We utilized an overexpression library of *C. albicans* genes cloned under the regulatable TET-ON promoter. Each construct was stably integrated at the RPS1 locus in a *C. albicans* chromosomal stability (CSA) reporter strain.[1] The CSA reporter strain contains two different fluorescent markers integrated at the same allelic locus of two homologs of chromosome 4: Chr4a and Chr4b. The resulting library was used to measure increased genome instability using flow cytometry-based analysis upon overexpression of individual ORFs. Genome instability was scored by measuring the frequency of loss of one of the fluorescent markers. The distinction between chromosomal loss events and non-chromosomal loss events was made using a third fluorescence marker present at the opposite arm of chromosome 4b.

Results: Out of the 532 *C. albicans* ORFs screened, five genes upon overexpression exhibited an increased genome instability. Two of these genes increased genome instability primarily by chromosome loss, while the remaining three exhibit genome instability due to non-chromosomal loss events. We identified one phylogenetically restricted gene, CSA11, present only in the CTG clade species of Ascomycota, with a previously unknown function in genome stability. CSA11 is important for cell cycle progression. Overexpression of CSA11 significantly increased the rate of erroneous chromosome segregation leading to aneuploidy.

Conclusion: We identified a phylogenetically restricted gene, CSA11, whose overexpression resulted in chromosome mis-segregation leading to aneuploidies. Further characterization and understanding of the regulatory mechanisms of these Candidate genes may reveal unknown pathways for maintaining genome stability and drug resistance. These genes may also serve as novel targets for developing antifungals.

Source:
Jaitly P, Legrand M, Das A, Patel T, Chauvel M. et al, A phylogenetically-restricted essential cell cycle progression factor in the human pathogen *Candida albicans*. 2021; bioRxiv: 2021.2009.2023.461448.

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Evaluation of the efficacy of fumigation practices on the mycological flora in the Orthopedic Operation theatre environment of a tertiary care hospital

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Objectives: To determine the pre and post-fumigation prevalence of fungi in the Orthopedic Operation Theatre of a tertiary care hospital and characterization of fungal isolates.

Method: This is a prospective environmental, analytical study conducted from July 2021 to January 2022. Pre- and post-fumigation samples were taken from Ultra and Modular OT of Orthopedic department every week by passive methods of air sampling using Gravity sedimentation method (1/1/1 scheme) on SDA and also by surface sampling using swabs. The fungal load of air was measured by calculating the number of CFU per cubic meter (CFU/m3) of air by Omeliansky formula. Fumigation of OT was done with a complex formulation of stabilized hydrogen peroxide 11% w/v with silver nitrate solution 0.01% (Baccishield®).

All surface samples were inoculated on SDA with chloramphenicol and all plates were incubated at 22°C and 37°C. The isolates were identified by using standard mycological procedures. Statistical analysis was done using a T-test.

Results: Out of 19 surface sampling, fumigation was found to be 100% effective only on 3 occasions (15.79%) in Ultra OT and on 7 occasions (36.84%) in Modular OT. In air sampling ≥50% reduction was found in only 4 samplings (21.05%) in Ultra OT and 10 samplings (52.63%) in Modular OT. The counts were much more than the WHO OT permissible limits. A total of 16 species of fungi were isolated belonging to 11 genera.

The most common isolate was *Aspergillus niger*, followed by sterile *hyalohyphomycetes*, *A. flavus*, *Cladosporium* spp., *Curvularia* spp., *Bipolaris spicifera*, *A. fumigatus*, etc. in both Ultra and Modular OT.

Additionally, *Exophiala* spp. and *Fonsecaea* spp. were isolated in Ultra OT.

The concentrations of fungi in Ultra OT before and after fumigation were in the range 22.11-58.97 CFU/m3 and 7.37-51.59 CFU/m3, respectively. Whereas, in Modular OT, the range was 14.74-36.86 CFU/m3 and 7.37-29.48 CFU/m3, respectively. Percentage reduction of fungi following fumigation with Baccishield varied from 0% to 75% both in Ultra OT and Modular OT. The statistically correlated *P*-value from pre- and post-fungal concentrations in Ultra and Modular OT were found to be .002 and .0002 respectively which was found significant by T-test, albeit ineffective as per standards.

Conclusion: In corroboration with our findings, Baccishield has been reported to be less effective even by other researchers. Hence, this needs to be replaced by more effective fumigants. The ineffectiveness of fumigation in Ultra OT was most probably due to the lack of HEPA filters and not strictly following up of aseptic protocols. In Modular OT improper maintenance of ACs and lack of periodic cleaning up of HEPA filters may be contributing factors.

Hence, implementing more stringent, frequent, and comprehensive disinfection and cleaning procedures, educating and motivating the health care personnel can help to improve the air quality of OTs that may aid in reducing post-operative infections.