P420
Fungal detection by means of HCR using 2D-Covalent Organic Framework Nanosheet
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The timely diagnosis of fungal infections is of prime importance for prescribing appropriate anti-fungal drugs. Current methods for fungal diagnosis involve culture-based methods, antibody-based direct detection using lateral flow assays and PCR.
In the present work, we derived a non-thermal amplification using 2D-Covalent Organic Framework (COF) nanosheet for the detection of fungal DNA.
Objectives: (1) Validation of exfoliated 2D COF Nanosheet as an efficient DNA detection tool via Hybridization chain reaction (HCR) triggered fluorescent assay. (2) Sequence removal and probe generation of fungal sample and detection of extracted target DNA via fluorescent assay.
Method: A novel COF was synthesized and characterization was done using FTIR, BET, TGA, XRR, and SEM. Probes for the detection of fungi (Candida, Aspergillus, and Mucor) were designed using NUPACK software. HCR was monitored for different time and probe concentrations and standardized reaction was used for the detection of fungal DNA.
Results: FTIR, BET, TGA, XRR, and SEM confirmed the structure and formation of COF nanosheet. H1, H2 probes at a concentration of λnas and in presence of Target DNA (0.01μm) showed HCR reaction at 1.5 s. Fluorescence quenching was observed when probes were mixed with both, bulk COF and COF nanosheets but increased quenching was observed.
Conclusions: Fungal detection can be done by means of HCR using the COF nanosheet.

P421
Whole-transcriptome analysis of Sporothrix brasiliensis grown in mold- and yeast-inducing conditions
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Objectives: Sporothrix brasiliensis is an emerging Sporothrix species limited to Brazil capable of causing sporotrichosis in humans and animals, especially in cats. Like other pathogenic Sporothrix species, S. brasiliensis exhibits a temperature-dependent dimorphic switch and, therefore, able to undergo a reversible morphological transition (mold and yeast), in response to environmental thermal stimuli.
While dimorphism appears to be essential for virulence in Sporothrix spp, the molecular mechanisms involved in this phenomenon have not yet been fully elucidated.
In this study, we used the strain-specific RNA-Seq technique and bioinformatics analysis to investigate the transcriptome signatures associated with mold and yeast phases of S. brasiliensis. Furthermore, we generated an accurate version of the S. brasiliensis genome annotation in order to perform high-quality gene expression analyses and other functional or structural genomic studies.
Methods: The whole transcriptome of S. brasiliensis AYCC-MYA-4251, grown in both yeast-inducing (YPD medium at 37°C) and mold-inducing (YPD medium at 25°C) conditions, was sequenced in this study. High-quality RNA was used to prepare Illumina TruSeq Stranded mRNA-paired-end sequencing libraries (2 × 150 bp). Next, the sequencing was performed using the HiSeq 2500 platform. A total of three biological replicates were sequenced for each condition.
Before transcriptome assembly, adapters and low-quality reads (Phred score <25) were removed. The StringTie software was used to assemble the transcriptomes imported into the Apollo workflow to manually curate the genome annotations. Transcriptomes were investigated using TransDecoder and CPC2 programs to determine whether a gene was potentially protein-coding or non-coding. Finally, differential gene expression analysis between yeast and mold forms of S. brasiliensis was conducted using the edgeR package.
Results: Illumina sequencing resulted in a total of ~217 million reads. After quality filtering and trimming, ~99.5% of reads were used for downstream bioinformatics analysis. The updated S. brasiliensis genome annotation consisted of a total of 14,648 genes of which 10,285 protein-coding genes, 1,623 lincRNA, 140 miRNAs, and 22 sRNA.
Gene expression analysis revealed a total of 13,838 and 13,938 transcripts expressed in mold- and yeast-form, respectively. Of these, 196 and 207 were expressed exclusively in the mold and yeast phase, respectively. Moreover, a total of 6,092 genes (FDR <0.05) were differentially expressed between the two examined conditions. In particular, 3,482 of these genes were up-regulated in the yeast form (2450 coding, 970 non-coding), and 3182 genes in the filamentous form (2307 coding, 875 non-coding). The raw reads have been deposited into the MIAP database and are available under BioProject PRJNA644924.
Conclusions: The characterization of the whole-transcriptome of S. brasiliensis mycelial and yeast-like forms represents an essential starting point for investigating the molecular pathways and regulatory frameworks associated with these two morphological stages. Our results provide new insight into global gene expression profiles of S. brasiliensis, emphasizing the role of non-coding RNAs in its complete transcriptional network.
All transcriptome data have also been integrated into the “Sporothrix Genome Database” (www.sporotrichismycology.unimib.it) in order to expand the current knowledge of Sporothrix genomics and to allow a more in-depth structural exploration of S. brasiliensis gene models, including gene expression patterns related to its saprophytic and pathogenic lifestyles.

P422
EUCLID PCP Score 2022—an ECMR score derived from current guidelines to measure QA/QM of clinical Pneumocystis management
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Background: Pneumocystis pneumonia (PCP) is a life-threatening opportunistic fungal infection requiring complex clinical management. Guidelines assist clinicians but can be challenging to comply with.
Objectives: To develop a scoring tool that facilitates and quantifies adherence to guideline recommendations for PCP management. We reviewed current PCP guidelines and determined essential recommendations for diagnosis, treatment, and follow-up. These were weighted according to their strength of recommendation and level of evidence.
Methods: The EUCLID PCP Score 2022 consists of 22 items. For diagnosis, weight was given to bronchoalveolar lavage and immunofluorescence assays as the gold standard for sampling and analysis. Beta-2-microglobulin was considered of similar importance due to its high negative predictive value. Transbronchial/lung COOKIE and the addition of corticosteroids in respiratory failure got 3 points respectively. Furthermore, approaches to reduce length of stay and the use of aerosolized pentamidine was discouraged with 1 minus point. HIV-specific considerations such as the start of secondary prophylaxis were factored in as well.
Conclusions: The EUCLID PCP Score 2022 weight and aggregates results recommended for optimal management of PCP. It provides a tool for antimicrobial stewardship as well as for measuring guideline adherence but remains to be combined with patient outcomes.