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Fungal detection by means of HCR using 2D-Covalent Organic Framework Nanosheet
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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM
The timely diagnosis of fungal infections is a prime importance for prescribing appropriate anti-fungal drugs. Current methods for fungal diagnosis involve culture-based methods, antifungal susceptibility testing, and direct detection using lateral flow assays and PCR.
In the present work, we derived a non-antimicrobial amplification using 2D-Covalent Organic Framework (COF) nanosheet for the detection of fungal DNA.
Objectives: (1) Validation of oxidized 2D COF Nanosheet as an efficient DNA detection tool via Hybridization chain reaction (HCR) triggered fluorescent assay. (2) Sequence retention 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, XRD, and SEM. Probes for the detection of fungi (Candida, Aspergillus, and Mucor) were designed using NUCASk 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, XRD, and SEM confirmed the structure and formation of COF nanosheet. FL-H2 probes at a concentration of λem and in presence of Target DNA (0.01 μM) λexc derived HCR reaction at 1:3 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.

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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 linked 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 strain and is therefore able to undergo a reversible morphological transition (yeast and mold), in response to environmental thermal stimuli.
While dimorphism is necessary 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 strand-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 analysis and other functional or structural genomic studies.
Methods: The whole transcriptome of S. brasiliensis ATCC MYA-4621, grown in two 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) that were sequenced using the HiSeq-2500 platform. A total of 32 biological replicates were sequenced for each condition.
Before transcriptome assembly, adapters and low-quality reads (Quality-score <25) were removed. The StringTie software was used to assemble the transcriptome. The assembled transcriptome was compared to the Apolo webtool 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 cuffdiff package.
Results: Illumina sequencing resulted in a total of ∼217 million raw 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 14 644 genes of which 10 241 protein-coding genes, 4 329 lincRNAs, 140 tRNAs, and 22 rRNAs.
Gene expression analysis revealed a total of 13 838 and 13 938 transcripts expressed in yeast- and mold-form, respectively. Of these, 362 and 281 were expressed exclusively in the mold and yeast phase, respectively. Moreover, a total of 4 602 genes (FDR <0.05) were differentially expressed between the two examined conditions. In particular, 3420 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 MRA database and are available under BioProject PRJNA644624.
Conclusions: The characterization of the whole-transcriptome of S. brasiliensis yealy 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 insights 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 Genomic Database” (www.sporothrixgenomicdatabase.unimi.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 lifestyle.

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EQUAL PCP Score 2022—an ECRMM score derived from current guidelines to measure QUAITY 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.
Objective: To develop a scoring tool, that facilitates and quantifies adherence to guidelines 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.
Results: The EQUAL 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-D-fucan was considered of similar importance due to its high negative predictive value. Transbronchialultrasonography and the addition of four compartments in respiratory failure gets 4 points respectively. Alternative approaches received less points and the use of aerosolised pentamidine was discouraged with 1 minus point. HIV-specific considerations such as the start of secondary prophylaxis were factored in as well.
Conclusion: The EQUAL PCP Score 2022 weight and aggregate scores recommended for optimal management of PCP. It provides a tool for antimicrobial stewardship as well as for measuring guideline adherence but remains to be correlated with patient outcome.