Comparative Transcriptomic Profiling of Fusarium keratoplasticum Clinical Isolates with Contrasting Amphotericin B Susceptibility

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Fusarium species are notably resistant to most available antifungal drugs, including amphotericin B (AmB), a polyene antifungal. Among them, *Fusarium keratoplasticum* is a clinically relevant pathogen that causes severe, often fatal infections, particularly in immunocompromised individuals. Given this context, we investigated the transcriptional response of *F. keratoplasticum* to AmB exposure using long-read sequencing with Oxford Nanopore Technology on the MinION platform. Two clinical isolates with contrasting minimal inhibitory concentrations (MICs) for AmB (LMC7108.01, MIC > 32 µg/mL, and LMC7113.02, MIC = 2 µg/mL) were exposed to AmB for 4 hours. Sequencing libraries were prepared following the MinION protocol, and FASTQ files were generated using MinKNOW with high-accuracy basecalling. Files corresponding to each barcode were processed using Cutadapt. The resulting sequences were subjected to ribosomal RNA (rRNA) depletion using SortMeRNA. The processed sequences were aligned to the *F. keratoplasticum* reference genome and its corresponding annotation available at NCBI using Minimap2. Differentially expressed genes (DEGs) were identified using DESeq2, applying a significance threshold of adjusted *p*-value < 0.05 and a log2 fold change of ±1.0. Functional annotation of the DEGs was performed using eggNOG-mapper. Raw data processing was satisfactory. During the filtering stage, retention of high-quality sequences ranged from 99.98% to 99.99%. All barcodes achieved 100% retention, and the proportion of clean sequences without poly(A) tails exceeded 90% across all barcodes. rRNA depletion also exceeded 90%, indicating that the data are reliable and free from contamination. The average read length ranged from 479 to 625 bp, with a mean quality score not lower than 13.8. For isolate LMC7108.01, 14,934 genes were annotated, whereas for isolate LMC7113.02, 8,309 genes were mapped to the reference genome. Differential expression analysis revealed log2 fold changes ranging from 9.73 to −3.28 compared with the untreated control, encompassing both known genes and novel isoforms modulated by the drug. The data showed coverage and quality comparable to those reported in similar studies. These findings aim to identify potential molecular targets for the development of novel therapeutic strategies and to enhance our understanding of resistance mechanisms, particularly in *Fusarium* species.

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