Development of Fungal Mock Community Standards for Mycobiome Studies

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Introduction

To date, a significant amount of work has been performed on the human microbiome to evaluate its composition and influence on physiology; this research has led to additional studies on microbiomes localized at specific sites of the human body (eg, skin, oral, vaginal). Given that fungi are ubiquitous and live in symbiosis with the human body, researchers are now actively looking into the role of the mycobiome in human health and disease. Recent advancements in sequencing technologies have enabled the community profiling of fungi; however, the complexities associated with metagenomics sequencing analyses have posed significant challenges toward standardization. To address this need, ATCC® has developed genomic DNA and whole cell mock microbial communities comprising ten medically relevant fungal species mixed in even proportions. In this proof-of-concept study, we demonstrate the use these standards in evaluating DNA extraction and sequencing methods for mycobiome analysis.

ATCC® Mycobiome Standards

Table 1: Genomic DNA and whole cell mycobiome standards

ATCC® No.	Product Name	Description
MSA-1010™	Mycobiome Genomic DNA Mix	Even mixture of genomic DNA comprising 10 fungal strains (2 x 10^6 genome copies of each organism per vial)
MSA-2010™	Mycobiome Whole Cell Mix	Even mixture of whole cells comprising 10 fungal strains (2 x 10^6 cells of each organism per vial)

Table 2: The fungal strains selected for the genomic DNA and whole cell mycobiome standards were chosen on the basis of their relevance in the normal and atypical flora of the human mycobiome

ATCC® No.	Species Name	Genome Size (Mb)	Relevancy
MYA-4609™	*Aspergillus fumigatus*	28.8	Opportunistic, airborne pathogen that is responsible for fungal infections in immunocompromised patients.
10231™	*Candida albicans*	17.1	Commensal fungus of the oral cavity that can form biofilms on denture surfaces, leading to mucosal infections.
2001™	*Candida glabrata*	12.3	Commensal fungus of the oral cavity and human gut that can acquire resistance to azole antifungals, leading to infection.
208821™	*Cryptococcus neoformans* var. *grubii*	18.9	Responsible for cryptococcal meningitis in immunosuppressed patients.
MYA-4612™	*Malassezia globosa*	9.0	Part of the normal skin flora but can be responsible for skin diseases such as dandruff, dermatitis, and folliculitis.
201390™	*Saccharomyces cerevisiae*	12.2	Bakers' and brewers' yeast originating in food. Emerging pathogen in immunocompromised patients.
9533™	*Trichophyton interdigitale*	21.9	Can infect skin and nails to cause chronic infections such as athlete's foot and ringworm.
204094™	*Cutaneotrichosporon dermatis (Trichosporon dermatis*)	23.3	Emerging opportunistic agent of invasive fungal infections, particularly in severely immunocompromised patients.
10106™	*Penicillium chrysogenum*	32.5	Spore-former, less prevalent, but can be responsible for intestinal infection in immunosuppressed patients.
36031™	*Fusarium keratoplasticum (F. solani* complex*)*	48.6	Filamentous, opportunistic pathogen that causes fungal keratitis.

DNA Extraction Efficiency

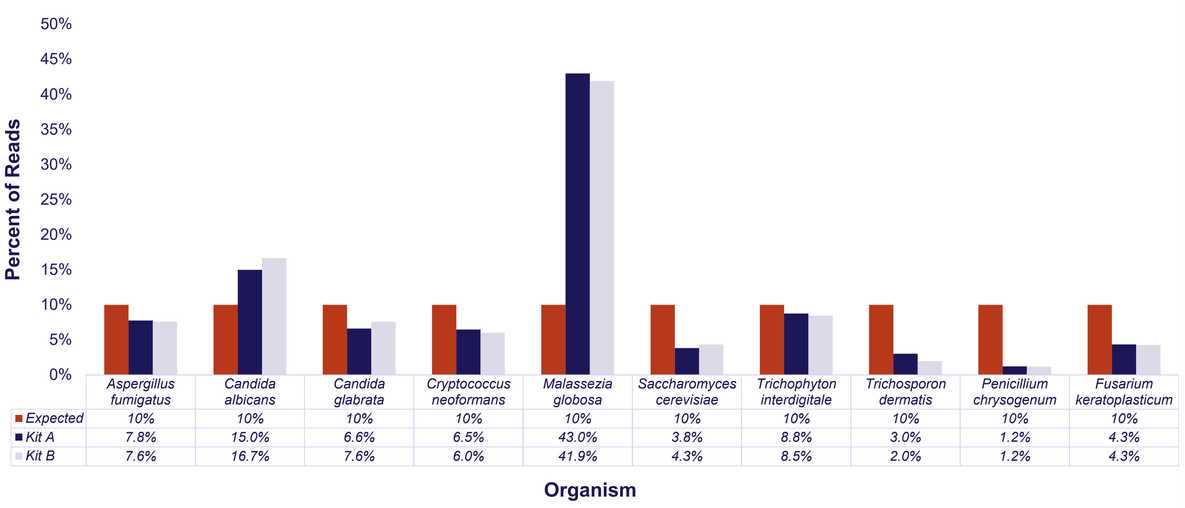

Figure 1: Whole cell mock mycobiome communities can be used to evaluate the impact of DNA extraction on the mycobiome analysis. DNA from the Mycobiome Whole Cell Mix (ATCC® MSA-2010™) was extracted via two different commercially available extraction kits. Shotgun data were generated on the Illumina® platform and were analyzed by using a commercially available database. The resulting data indicate that extraction methods can affect downstream microbiome analyses. The disproportionate results observed may be attributed to the diversity of organisms in the mix and the inefficient extraction of the organisms within the mix.

ITS and Shotgun Sequencing

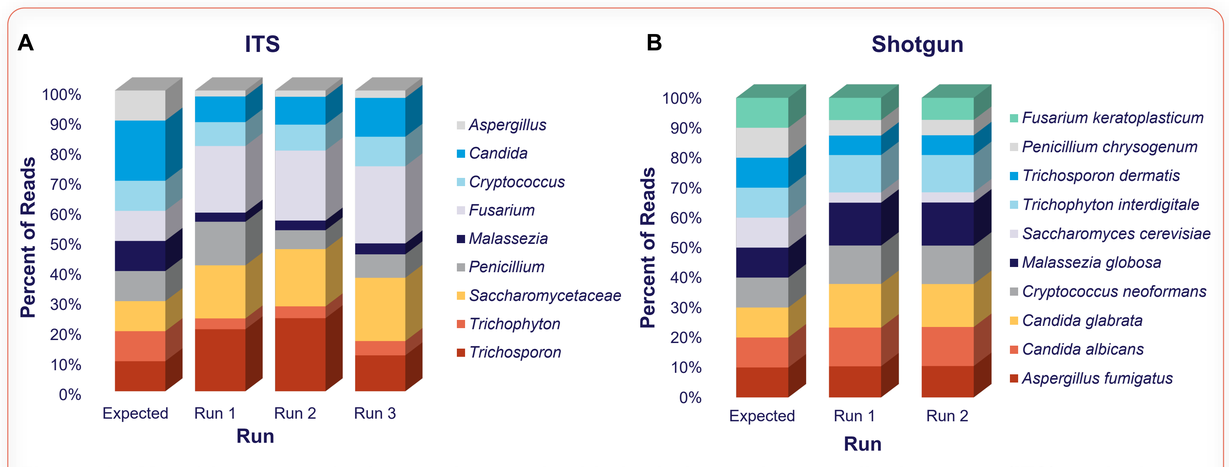

Figure 2: Mycobiome standards can be used with both internal transcribed spacer (ITS) and shotgun metagenomic sequencing assays. The Mycobiome Genomic DNA Mix (ATCC® MSA-1010™) was examined via (A) ITS and (B) shotgun metagenomic assays on the Illumina platform, and data were analyzed via a commercially available data analysis platform. The ITS analysis could profile all the fungi to genus level where as shotgun metagenomic assay could identify at species level. The results also demonstrated run-to-run variation when performing both sequencing assays.

Next-Generation Sequencing Technologies

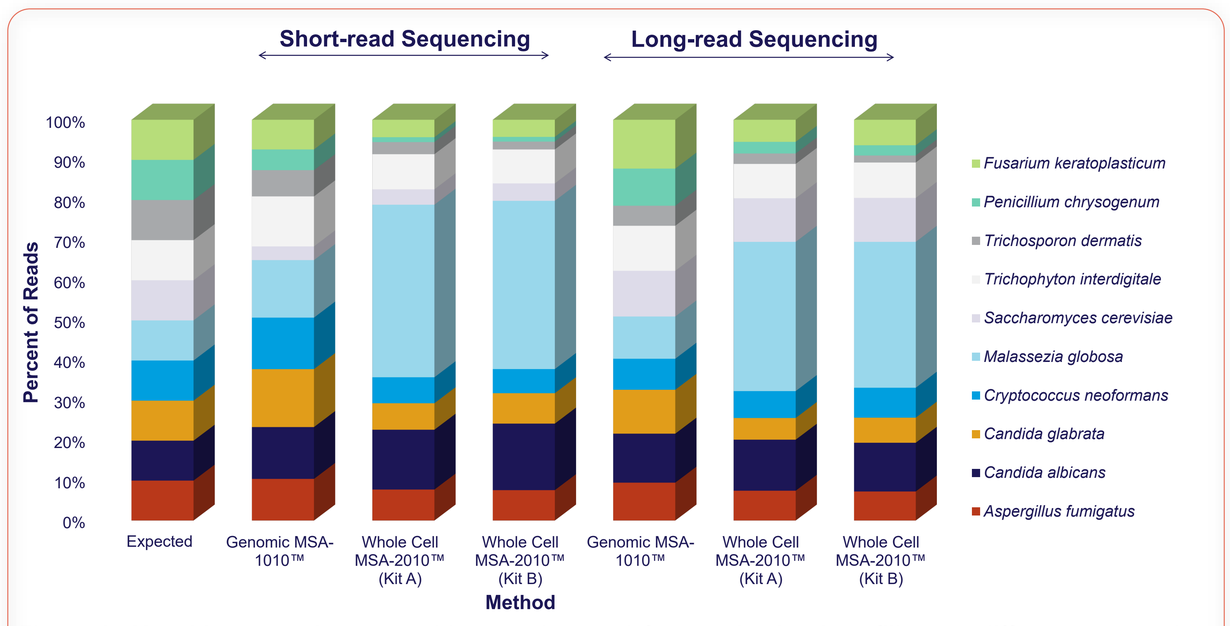

Figure 3: Mycobiome mock communities can be used for species-level profiling on different sequencing platforms. The Mycobiome Genomic DNA Mix (ATCC® MSA-1010™) and genomic DNA extracted from the Mycobiome Whole Cell Mix (ATCC® MSA-2010™) were evaluated via shotgun sequencing on a short-read sequencing platform (Illumina MiSeq™) and a long-read sequencing platform (Oxford Nanopore Technologies® GridION™). Genomic DNA from the whole cell mix was extracted via two different commercially available kits. The genomic mix produced results consistent with the expected percentage. The variation seen in data obtained from the whole cell mix can likely be attributed to differences in DNA extraction efficiency.

Data Analysis and Databases

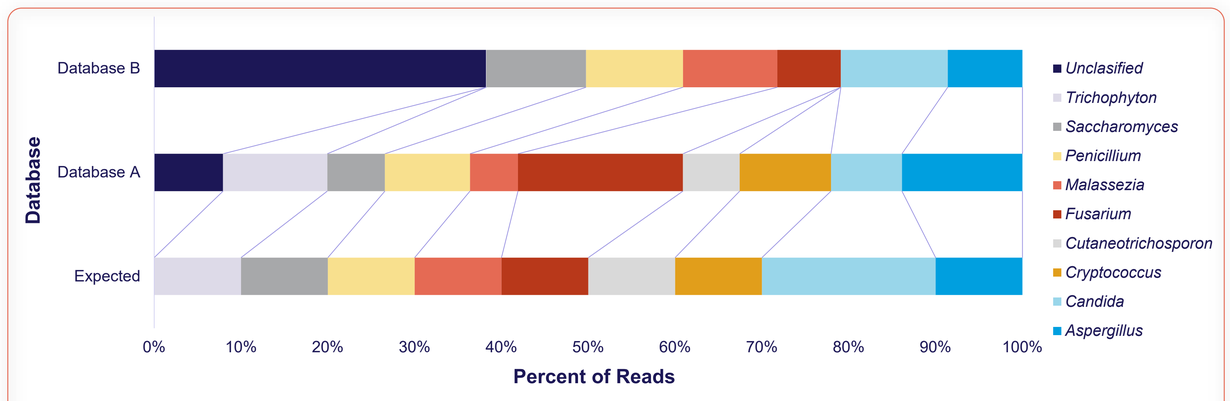

Figure 4: Mycobiome standards can be used to compare bioinformatics platforms. Data generated from sequencing the Mycobiome Genomic DNA Mix (ATCC® MSA-1010™) on the Illumina platform were analyzed via two different publically or commercially available bioinformatics databases. Our data demonstrate the potential problem of biases during the data analysis step.

Conclusions

This proof-of-concept study demonstrates the utility of mycobiome standards as controls for evaluating run-to-run variability and optimizing assay performance at each stage of the mycobiome analysis workflow.

- Whole cell standards can help identify biases introduced during DNA extraction and can be used as full-process controls.
- Genomic DNA standards can be used for comparing various library preparation methods and sequencing platforms.
- The data analysis for mycobiome profiling is challenging due to the lack of complete fungal reference genomes and the limited availability of analyses pipelines.

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