Fungal identification: How accurate and robust is your characterization strategy?

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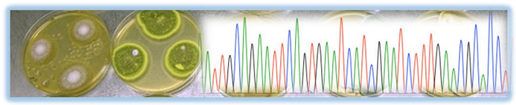
1 ABSTRACT

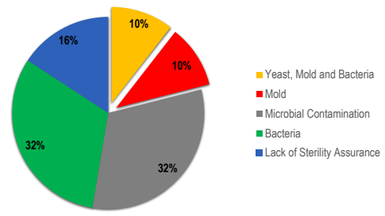
Despite the fact that product recalls due to fungal contamination are on the rise for non-sterile and sterile pharmaceutical products, fungal identification, especially for mold, has not been held to the same standards as bacterial. The increase in mold-related recalls, however, has resulted in increased scrutiny from the FDA with respect to properly characterizing fungal samples isolated during environmental monitoring activities and product testing. Even though accurate fungal identification is necessary to determine and track the contamination source, most pharmaceutical microbial testing relies on very subjective phenotypic identification kits. While a number of fungal identification tools are available to the pharmaceutical market, most of these tools tend to underperform since they do not support the broad range of microorganisms encountered in environmental monitoring programs. Here, we compare six commercial systems and their database coverage for fungal contaminants that are frequently encountered in environmental monitoring programs. As part of the study, we compared database coverage of two phenotypic, two proteotypic, and two genotypic systems. In addition, we also evaluated the utility of the genotypic target-regions, 28S rRNA and ITS, for their species resolution capabilities for frequently occurring filamentous fungal isolates and contaminants.



2 BACKGROUND

The FDA reported that for the year Oct 2017-Sep 2018, 30% of all drug recalls were due to microbial contamination. Of these recalls, 20% were indicated to have been due to a fungal contaminant and 48% were undefined contamination events. An accurate species-level identification is critical to reliably identify the source of contamination and to take appropriate corrective actions to prevent future incidents. The goal of this study is to evaluate six commercial platforms that are widely used in the industry for fungal identification for their database coverage of frequently isolated fungal contaminants from environmental monitoring (EM) programs.

Fig 1. Survey of FDA drug recalls during Oct 2017-Sep2018 – determining microbial contamination type



3 EVALUATION STRATEGY

Database comparisons

The study cohort included 237 unique fungal species belonging to 76 unique genera representing the top 85% of the frequently occurring fungal species (shown as FOO) identified at Charles River Laboratories (CRL). Filamentous fungi dominated the study cohort contributing 92% of all the identifications (Fig 2A). The study cohort also included samples that belonged to species complexes or groups (16%), where the identification to one species was not possible due to low sequence diversity between closely related species (species* in Fig 2B). Six commercial databases were evaluated for their database coverage for the frequently occurring fungal contaminants. The commercial databases tested included two genotypic (Accugenix® Fungal Library & platform A), two proteotypic (platform B & C), and two phenotypic (platform D & E) systems.

ITS2 vs. 28S (D2)

To test the utility of the different gene targets for fungal identification, 28S (D2) and ITS2 sequences were searched against the databases from platform A and the Accugenix® Fungal Library. The study cohort represented the gene sequences from FOO organisms comprising 21 known culture collection type strains and 82 unknown fungal samples.

Fig 2. Top 85% FOO fungal identification showing (A) organism type represented and (B) Taxonomy level confidence

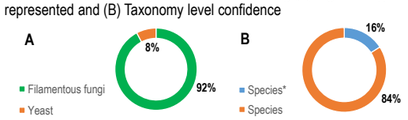
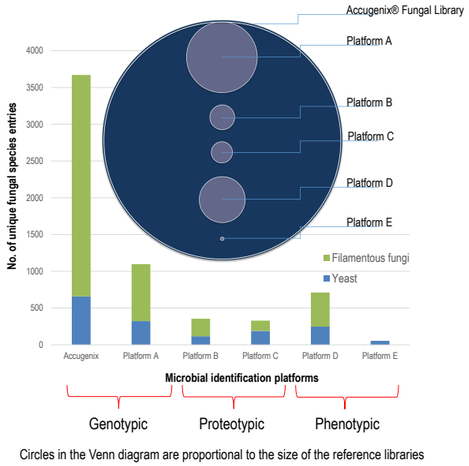


Table 1. Top 10 genera representing 85% FOO samples

Genus	No. of Species	Genus	No. of Species
Alternaria	10	Epicoccum	2
Aspergillus	39	Paecilomyces	3
Candida	8	Penicillium	42
Chaetomium	3	Pithomyces	3
Cladosporium	7	Rhodotorula	3

4 RESULTS

Fig 3. Fungal library database comparisons showing database size and fungal organism type represented in each platform



Circles in the Venn diagram are proportional to the size of the reference libraries

Fig 4. Library database comparisons for the top 85% FOO species identified at CRL

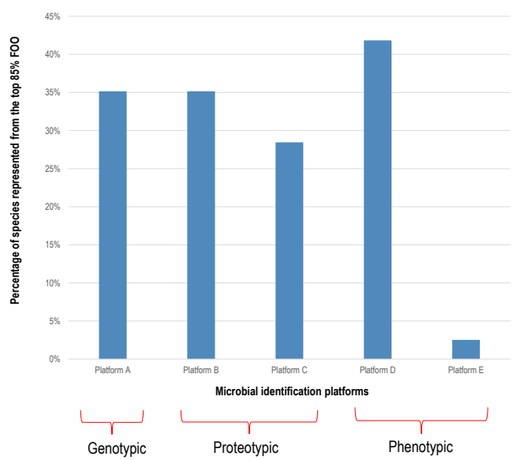


Fig 5. Comparison of known culture collection type strain sequences searched against D2 (platform A) and ITS2 (Accugenix® Fungal Library) databases

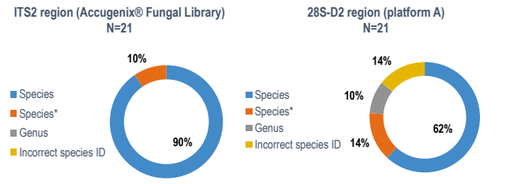
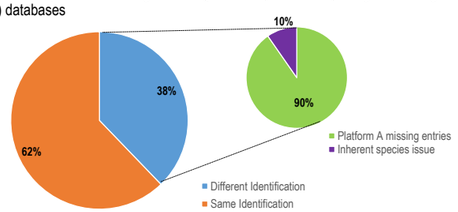


Fig 6. FOO samples searched against D2 (platform A) and ITS2 (Accugenix® Fungal Library) databases



5 CONCLUSIONS

- Evaluation of the frequently occurring fungal samples identified at CRL showed that 92% are filamentous fungi and only 8% are yeast (Fig 2A).
- The commercial platforms (A, B, C, D, and E) are more geared towards clinically significant organisms, and thus do not support the broad range of microorganisms encountered in manufacturing facilities.
- Comparisons of the five commercial databases (A, B, C, D, and E) indicated that less than 50% of relevant fungal species – the top 85% of our most frequently occurring organisms – were present in the commercial library databases (Fig 4).
- ITS2 vs. D2 comparisons of known culture collection type species' sequences using commercial platforms (Accugenix® Fungal Library and Platform A) supported a higher level of species resolution using the ITS2 database (Fig 5).
- A comparison of unknown fungal samples showed discrepancies in identification between the two genotypic platforms. A closer evaluation of the results showed that these discrepancies was mainly due to missing relevant species entries in the Platform A database (Fig 6).

6 ACKNOWLEGMENTS

We thank Microgenetix, (now part of Charles River Laboratories) for helping with D2 sequence analysis for this study.