

ACCURATE IDENTIFICATION OF MICROBIAL CONTAMINANTS: CASE STUDY OF *ASPERGILLUS* AND *PENICILLIUM*

Cindy Serrato Zavala, Ryan Cox, Sujan Timilsina, Bindhu Verghese, Charles River Laboratories, Newark, DE

1 ABSTRACT

Mold contamination in manufacturing facilities can be a serious issue for pharmaceutical and life science companies. Not only does it endanger patient safety, but it can also cause product recalls, financial and reputational harm, and regulatory ramifications. Rapid, accurate, and reliable microbial identification provides understanding of the environmental quality and acting on to avert risk and contamination. In this study, we analyze two fungal genera: *Aspergillus* and *Penicillium* that are frequent fungal contaminants, producing mycotoxins and with the ability to cause human infections. Identification of organisms within these genera can be tricky across different platforms due to species diversity. A total of 14,687 *Aspergillus* and 14,827 *Penicillium* samples were identified across Accugenix® global labs in 2023, 23.5% of these samples were identified using a proteotypic method and the remaining by genotypic. More than 100 unique species groups were identified, for each genus, during the process. We further evaluate the potential of these organisms to be conclusively identified using three different identification platforms and compare species diversity across multiple databases. The distribution of these critical organisms across Accugenix® global labs emphasizes the necessity for robust cross functional approach to identify the organisms confidently strategically nullify any contamination risks.

2 BACKGROUND

- Identifying fungi through phenotypic methods, such as colony morphology, introduces subjectivity into the final identification given and ultimately gives a less accurate and precise identification. Figure 1 shows how the colony morphology of *Aspergillus brasiliensis* ATCC 16404 can vary in different growth media plates despite all other growth conditions, ex: culture age, temperature, humidity, etc., being the same.

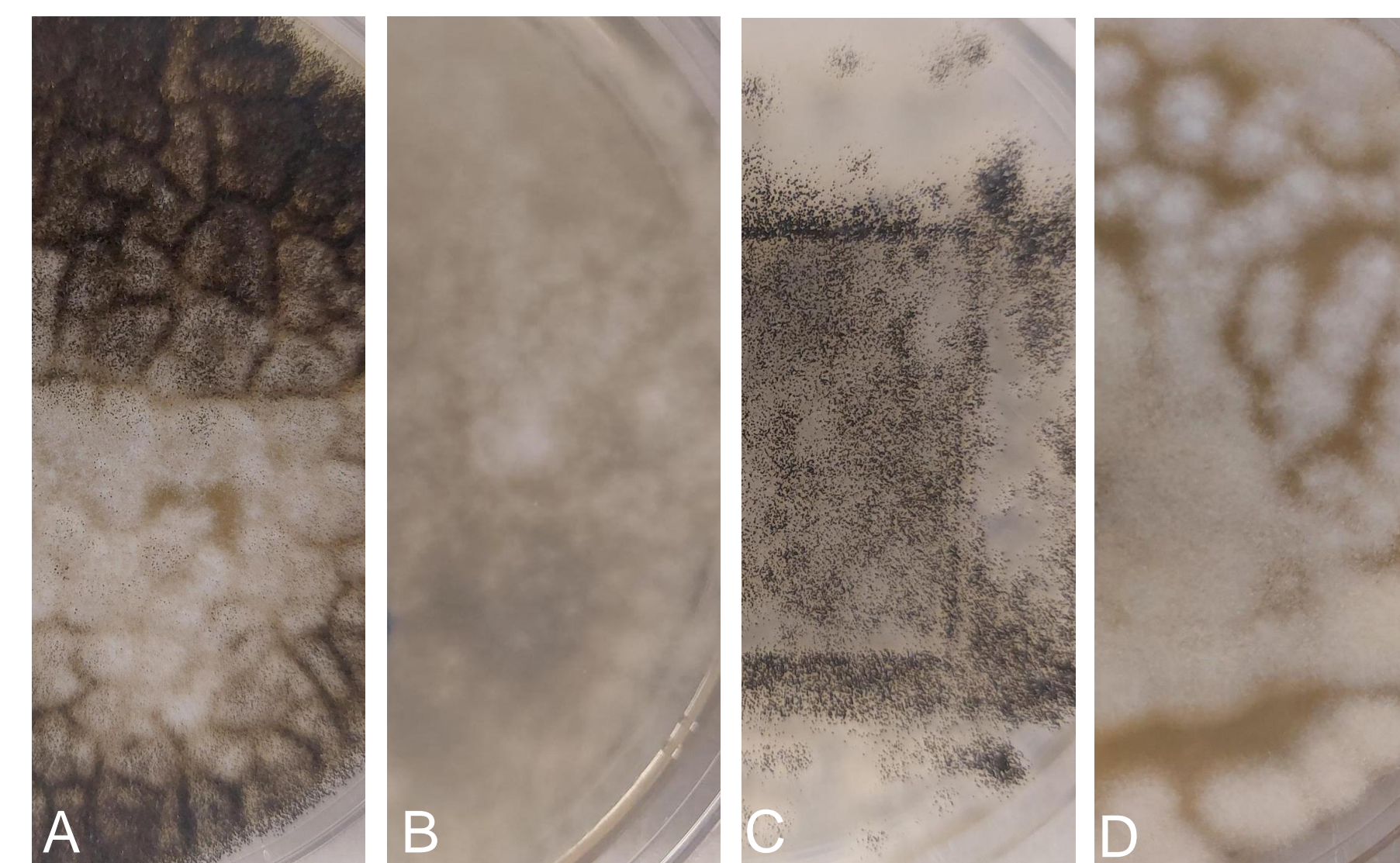


Figure 1. *Aspergillus brasiliensis* ATCC 16404 grown at 25° C for 5 days on (A) Emmons Sabouraud Dextrose Agar, (B) Tryptic Soy Agar, (C) Conidia IDFP, and (D) Yeast Malt Agar. *Aspergillus brasiliensis* is a known contaminant and therefore a recommended compendial testing organism.

- Proteotypic and genotypic measures provide a rapid, robust, and precise method of identification for environmental monitoring and regulatory purposes.
- This study centers on two major fungal genera *Aspergillus* and *Penicillium*, known contaminants, where we compare their identification frequency among customer samples, and the species diversity available in two additional commercially available microbial identification platforms.
- We further evaluate the chances of identifying different species within these two genera while using different database libraries.

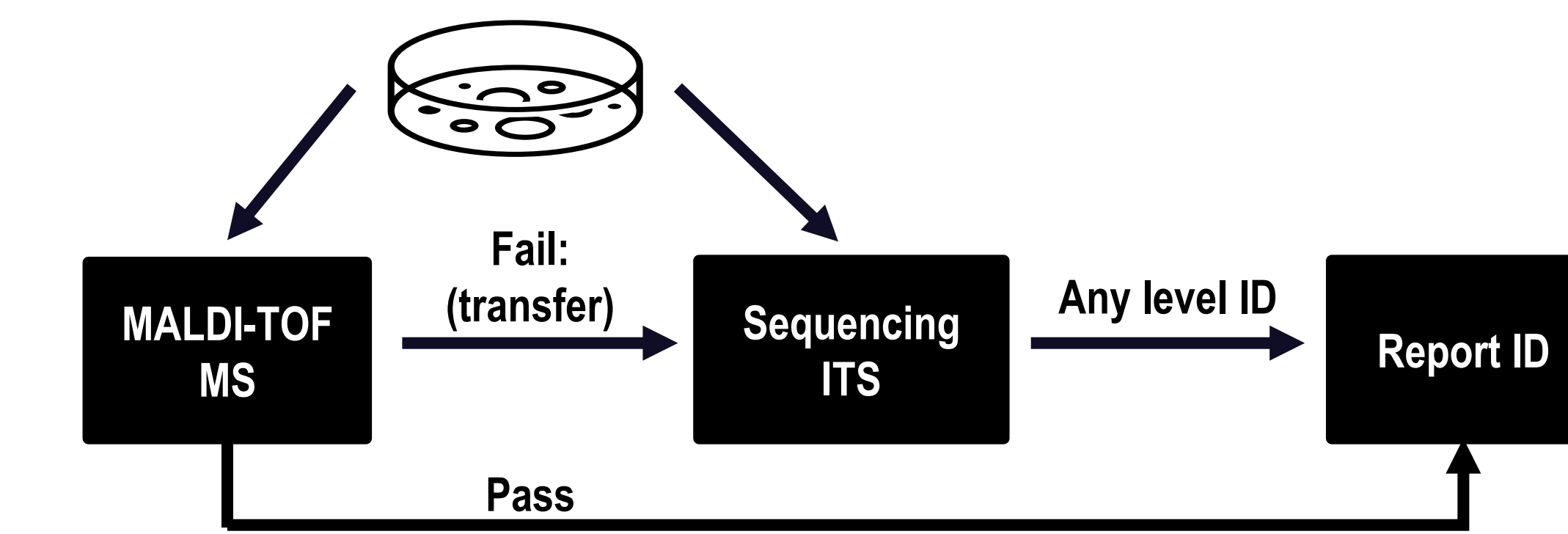


Figure 2. Workflow of Accugenix® identification services. If a sample is submitted for MALDI-TOF MS identification and does not provide a result, the sample is sequenced to provide an identification.

3 RESULTS

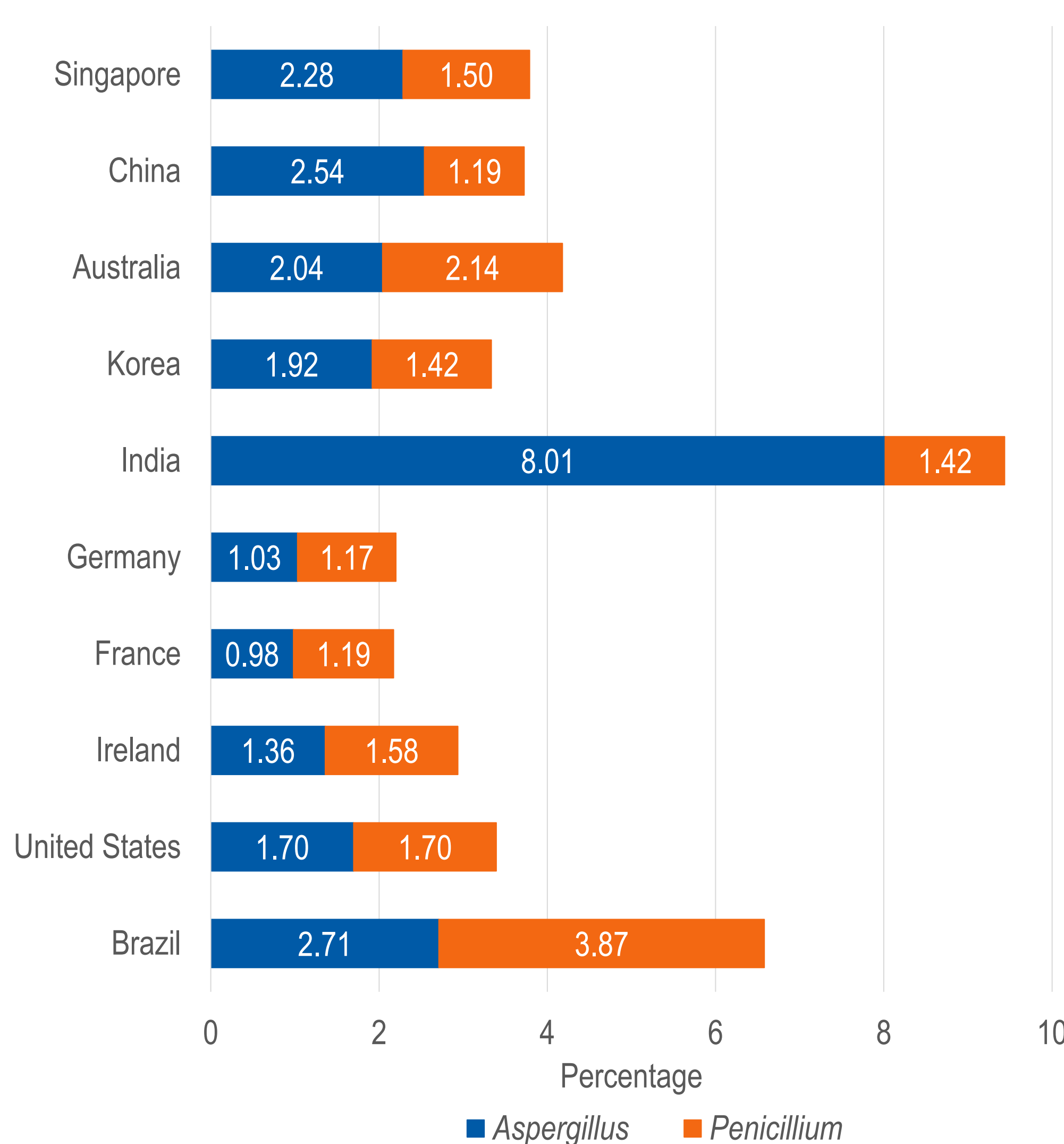


Figure 3. Frequency of identification of the two most routinely identified fungal genera *Aspergillus* and *Penicillium* in 2023 across Accugenix® global labs.

- Aspergillus* and *Penicillium* are among the most common fungal contaminants; they share a significant portion of species identified through the Accugenix® global labs (Figure 3). The global distribution of these organisms shows the relevance of monitoring them in different production environments.
- Table 1 shows several *Aspergillus* and *Penicillium* species, objectionable organisms and/or organisms of regulatory relevance, commonly identified through the Accugenix® platform, and their presence in other commercially available platforms. Routine identification of organisms are impacted by various factors such as presence or absence of organisms, diversity of strains, and quality of data in the reference libraries.
- Aspergillus* and *Penicillium* both consist of more than 250 known species. Figure 4 shows the number of species captured in different commercially available databases for these two genera.

	Accugenix® Database	Database B	Database C
<i>Aspergillus clavatus</i>	+	+	-
<i>Aspergillus brasiliensis</i>	+	+	+
<i>Aspergillus flavus</i>	+	+	+
<i>Aspergillus fumigatus</i>	+	+	+
<i>Aspergillus shendawei</i>	+	-	-
<i>Aspergillus terreus</i>	+	+	+
<i>Penicillium brevicompactum</i>	+	+	+
<i>Penicillium chrysogenum</i>	+	+	+
<i>Penicillium citrinum</i>	+	+	+
<i>Penicillium cyclopium</i>	+	-	+
<i>Penicillium decaturense</i>	+	-	-
<i>Penicillium steckii</i>	+	-	-

Table 1. Database comparison showing organisms of interest, frequently observed in quality control testing and/or product recalls. Database B and C refer to commercially available proteotypic databases.

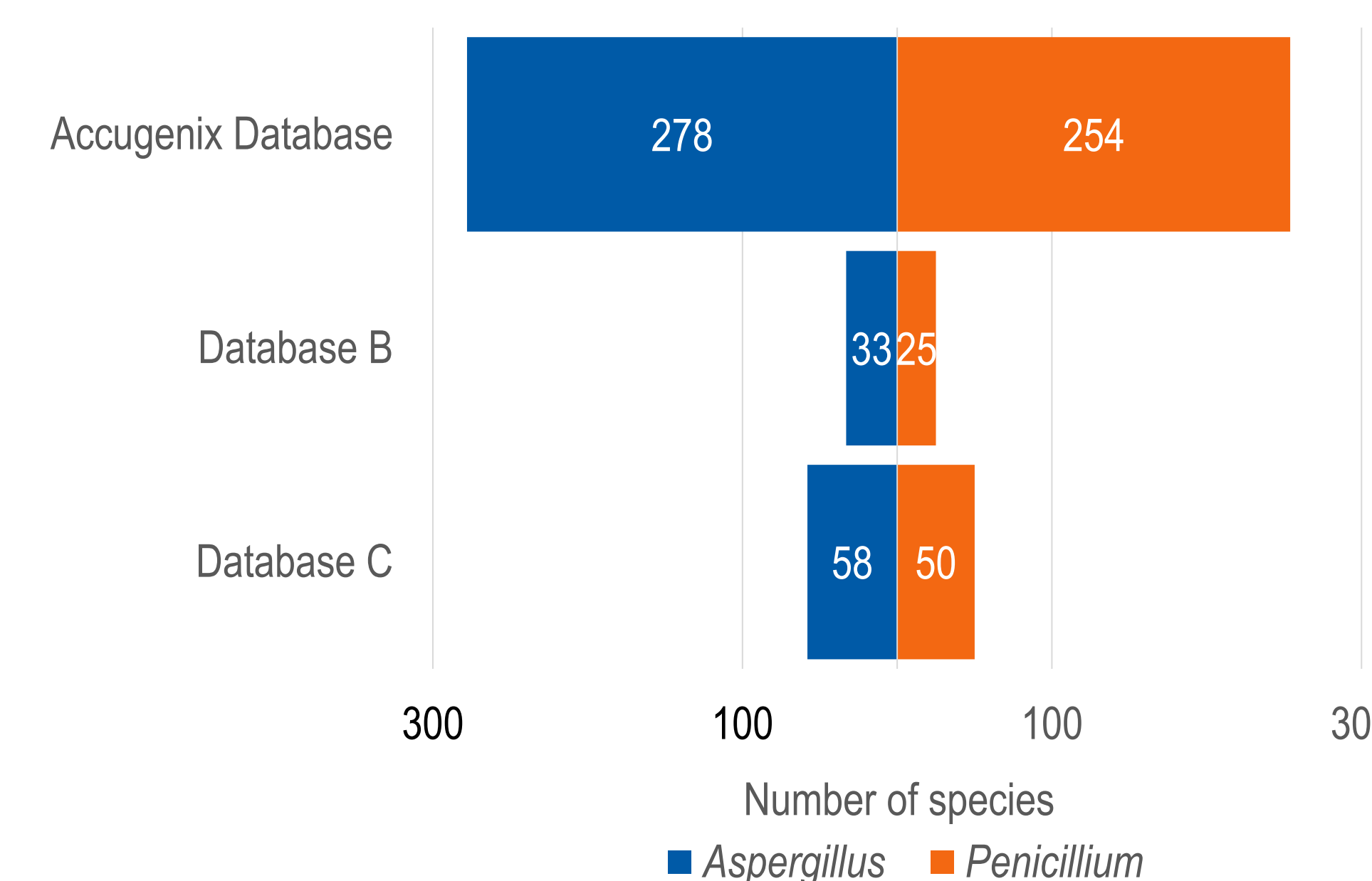


Figure 4. Database comparison showing number of *Aspergillus* and *Penicillium* species in different commercially available platforms. Databases B and C refer to commercially available proteotypic databases.

4 CONCLUSION

- Aspergillus* and *Penicillium* are two large genera with spore-forming capabilities making them ubiquitous, and therefore a common contaminant. Species of both genera are known to produce mycotoxins, leading to health hazards, and thus posing a regulatory risk. Both genera have been involved in recalls recently and regulatory bodies have updated mandates for routine monitoring to limit contamination from similar risks.
- Database used for comparison plays a central role in organism identification. Comparing to a taxonomically robust and well-encompassing database provides accurate and up-to-date identification.
- Proteotypic approach, in combination with relevant organisms in the database utilized, supports rapid and accurate identification for Environmental Monitoring programs.

5 WORKS CONSULTED

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