

Navigating Risk-Based Approaches with Annex 1: Prevalence of Spore-forming Microorganisms and Molds Recovered During Routine Environmental & Process Monitoring



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1 INTRODUCTION

The current guidelines established by Annex 1 require the quality of sterile medicinal products to be assured through controlled processes. Therefore, environmental monitoring (EM) is an important part of the production of aseptic products, as evident by entire section (9) being dedicated to this practice within Annex 1. Furthermore, within section 9 Annex 1 maps out:

...Consideration should also be given to the identification of microorganisms detected in grade C and D areas (for example where action limits or alert levels are exceeded) or following the isolation of organisms that may indicate a loss of control, deterioration in cleanliness or that may be difficult to control such as spore-forming microorganisms and moulds and at a sufficient frequency to maintain a current understanding of the typical flora of these areas.¹

This study evaluates bacterial and fungal diversity from samples identified from three global regions (See regions A,B,C in Figure.1) over a one-year period starting from January 1st 2022 – January 1st 2023. To evaluate common, often challenging microflora for contamination control, over 500,000 global samples were identified by sequencing 16S and ITS2 gene-regions and using MALDI-TOF. The reference libraries used in this study were continuously curated and updated to reflect taxonomic changes and inclusion of novel organisms encountered in sterile and non-sterile manufacturing environments. Microorganisms are the contaminant within contamination control thus making accurate species identification paramount. Without knowing predominant resident microflora within a facility neither the root cause in product investigations nor how objectionable an organism is to the product can be known.

2 MATERIALS AND METHODS

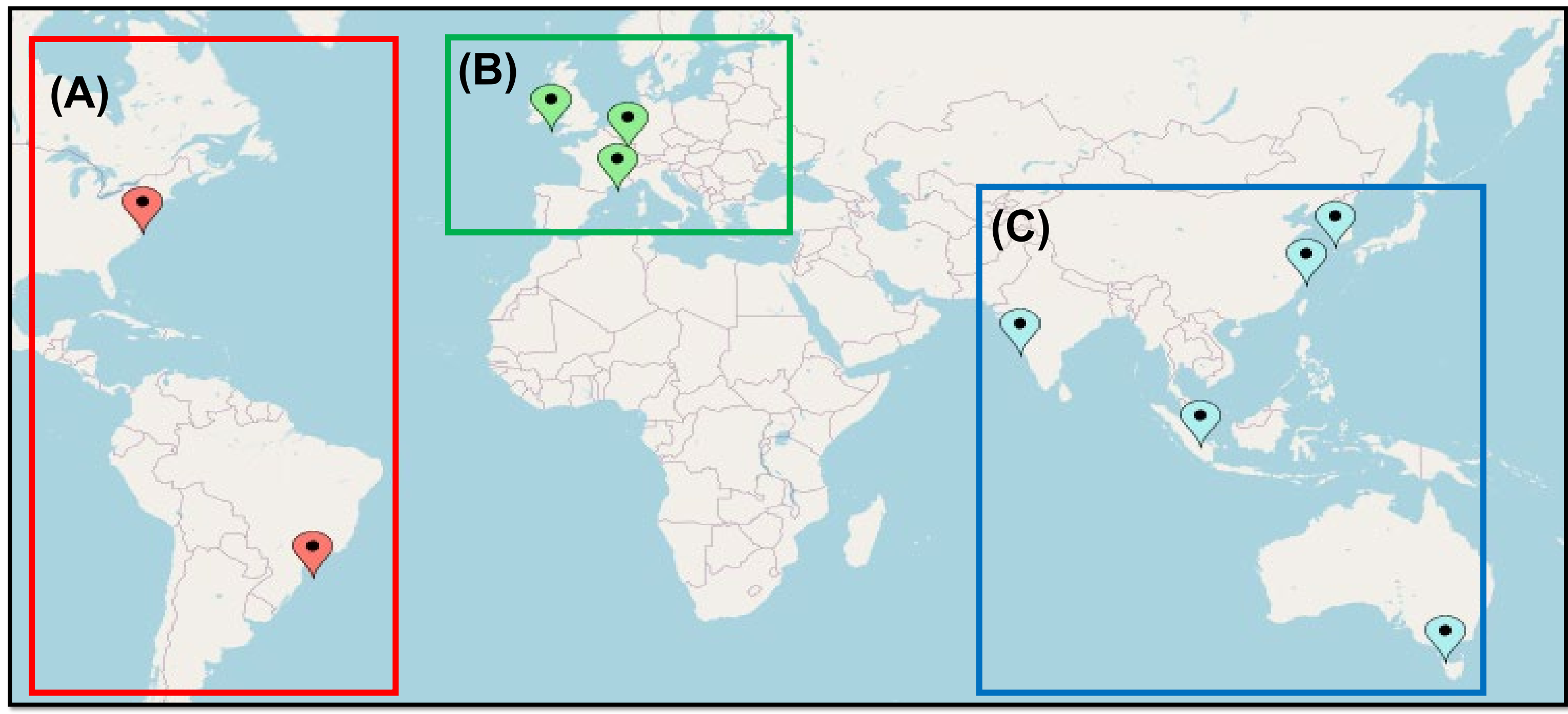
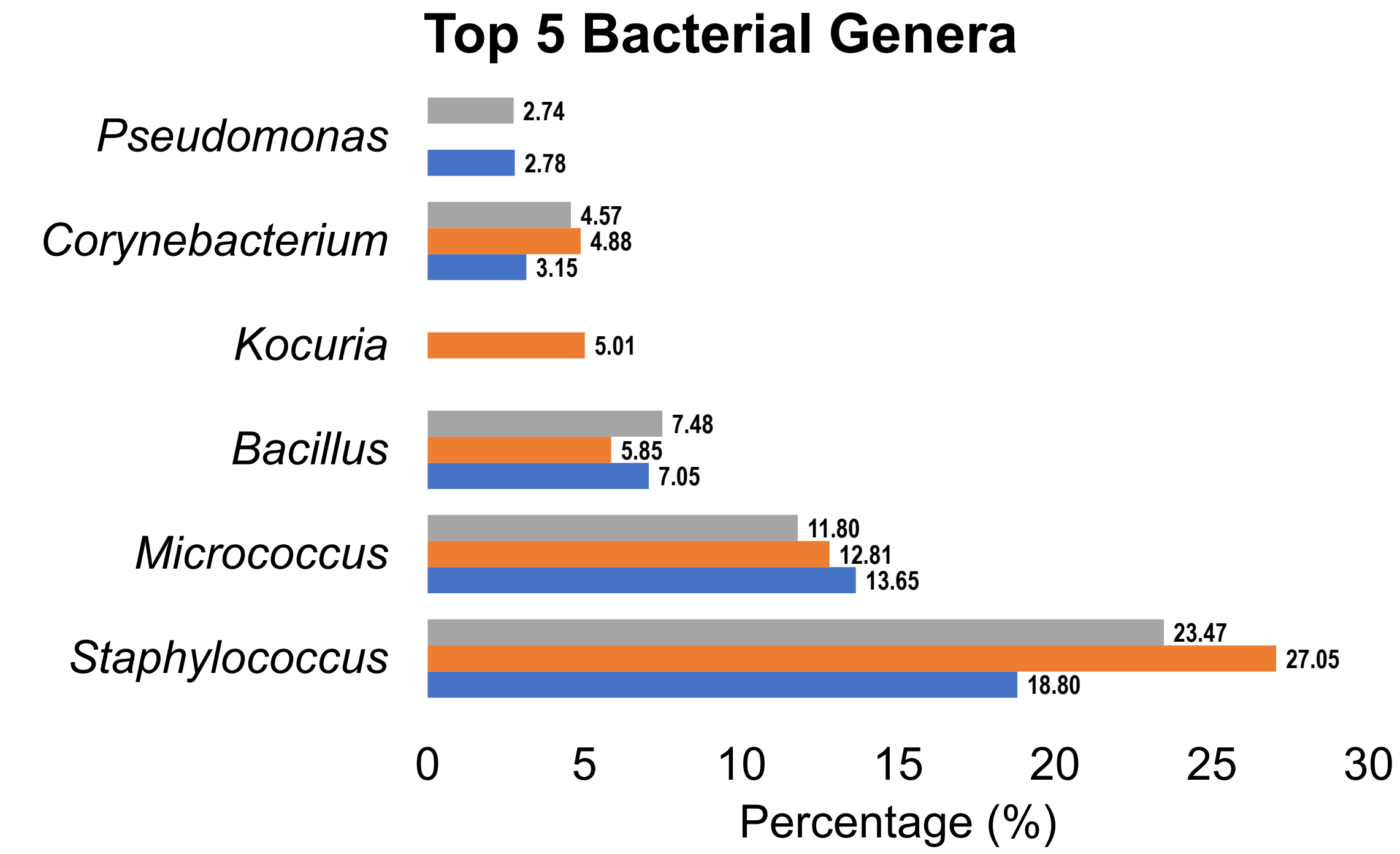
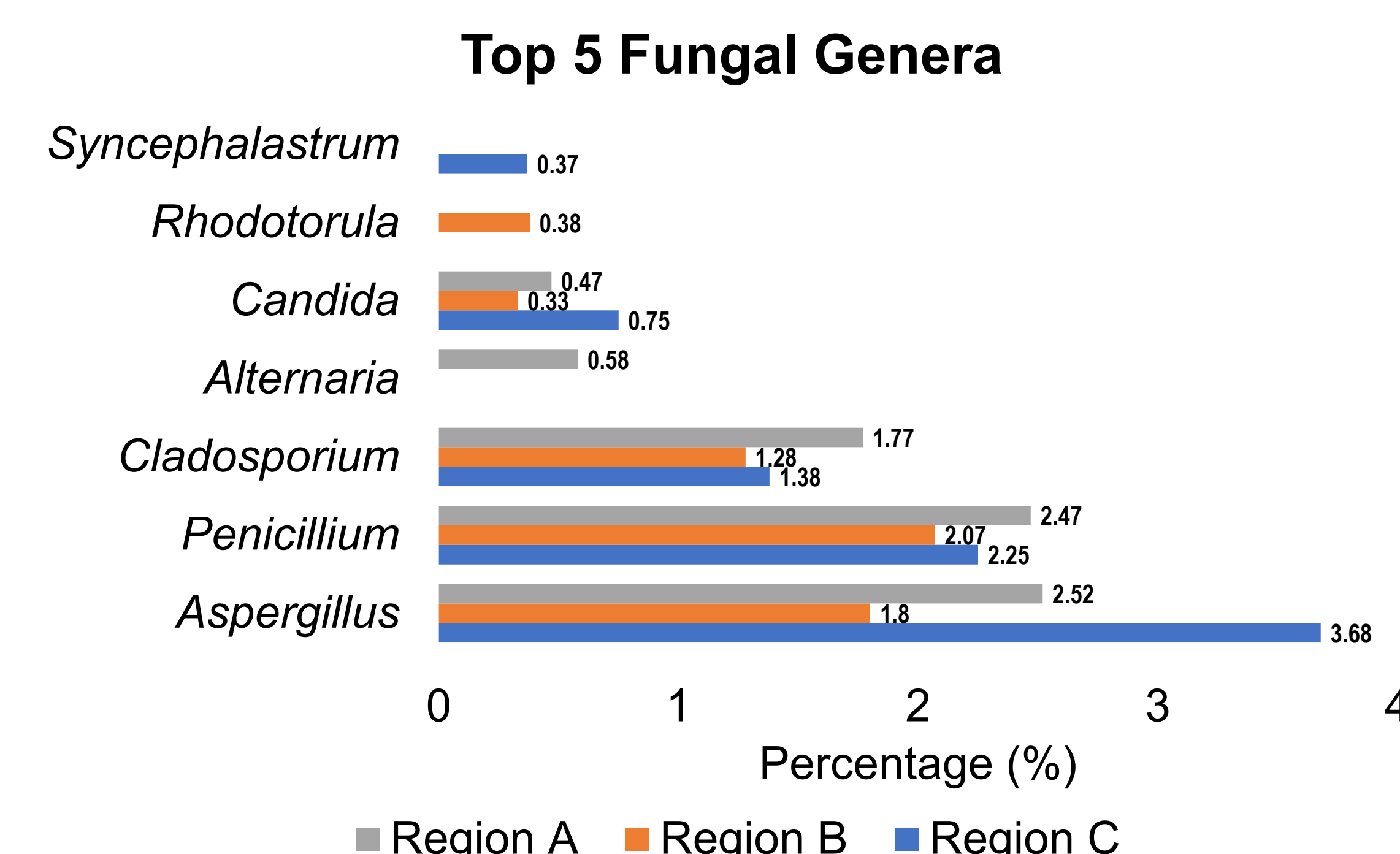


Figure 1. Sample identifications performed using Accugenix® services in 2022 from Brazil, and USA (Region A), France, Germany, and Ireland (Region B), Australia, China, India, Singapore, and South Korea (Region C). Samples obtained reflect isolates from bio- pharmaceutical, medical device, nutraceutical and personal care facilities. Regions were determined by geographic relevance to observe global microorganism identification patterns. A total of 574,862 samples were identified to a species level utilizing MALDI-TOF, 16S rDNA and ITS2 gene sequence data 373,977 (A), 158,074 (B), and 42,811 (C). The analyses performed for this study aimed to highlight the regularity of difficult to control microorganisms, particularly spore-forming bacteria and molds, recovered from diverse sterile and non-sterile manufacturing environments.

3 RESULTS



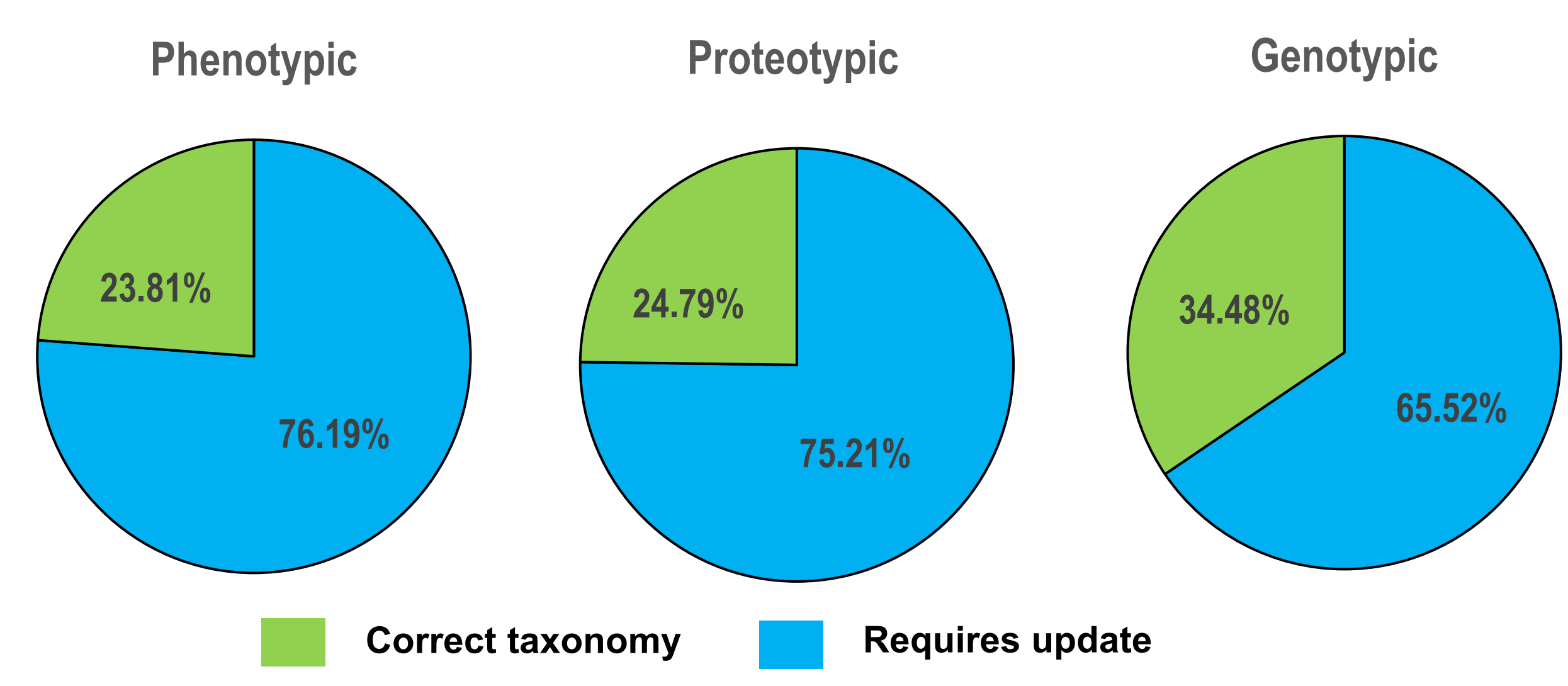
- *Staphylococcus*, *Micrococcus*, and *Bacillus* are the top three most frequently identified Genera across all regions.
- While bacteria are more frequently identified, when considering the frequency of spore-forming, or mold microbial species, these difficult to control organisms make up:
 - Region A - 35.88%
 - Region B - 29.07%
 - Region C - 34.80%



- When considering the top 50 most frequently identified genera per region, spore-forming bacteria or filamentous fungi make up:
 - 38/50 of identifications in Regions A & B
 - 37/50 of identifications in Region C
- Regardless of ID method, the library that is used to identify microbial contaminants should be current and robust (Figure 3).
 - Identifying contaminant organisms accurately and consistently is critical and assures that an environment is in a state of control.

Figure 2. Percentage of top 5 bacterial and fungal genera identified from global Accugenix® services. Microbial diversity did not show significant variation with geographic location.

(3A) Status of *Bacillus* taxonomy in different databases



Taxonomic Status of *Bacillus* since 2020

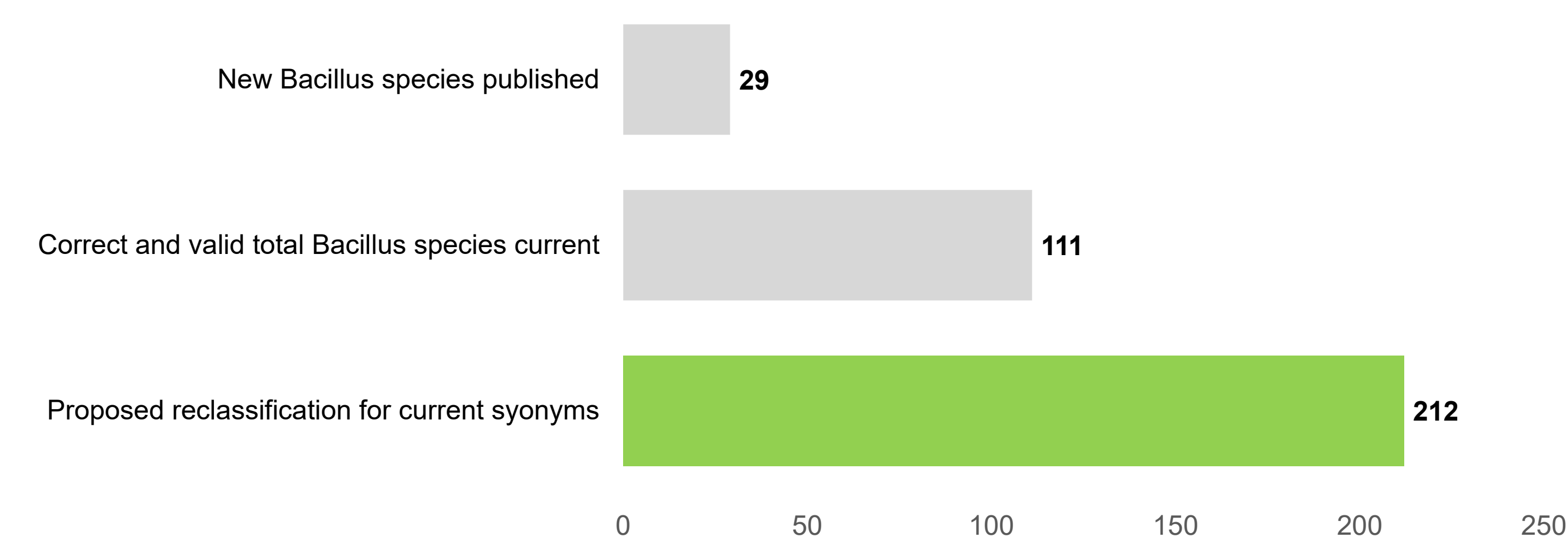
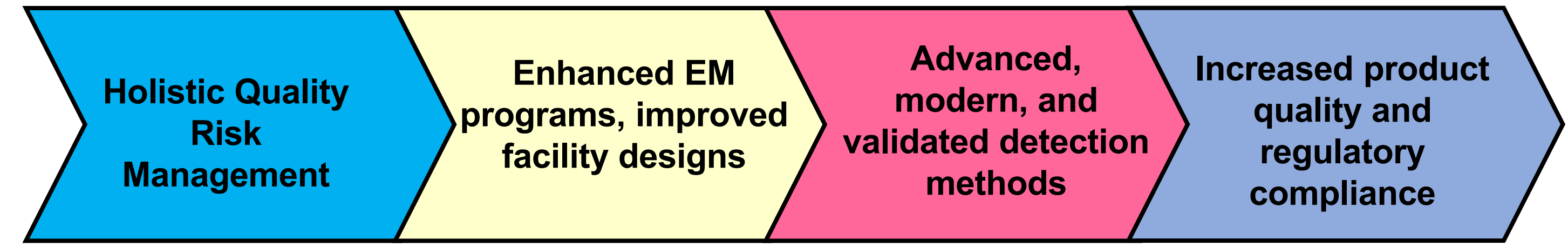


Figure 3. (A) Comparison of *Bacillus* microbial taxonomy determined by three major commercial microbial identification platforms utilizing either phenotypic, proteotypic, or genotypic methods. Most of the bacterial species identified via these commercial platforms compared in this study have been reclassified emphasizing the necessity of using an updated database. (B) Recently proposed reclassifications within *Bacillus* genus (since 2020). The number of reclassifications and new organisms highlights the importance of up-to-date microbial database used for comparisons. Correct microbial identification is also essential to implement appropriate corrective and preventative actions for contamination control.

4 CONCLUSIONS

- The results of this study indicate filamentous fungi and spore-forming bacteria are ubiquitous and routine environmental monitoring will be effective to maintain the regulatory standards and proper production procedures.
- Annex 1 states monitoring systems should include scientifically sound, modern methods that optimize detection of contamination.²
 - ❖ Using outdated or minimally diverse libraries more often yields no identification or misidentification leading to improper contamination control strategies or disinfection approaches. Knowing what species is in a facility will ensure proper disinfection actions can be executed.
 - ❖ Phenotypic methods, especially when applied to fungal organisms, are challenged by taxonomic variability and morphological subjectivity; adoption of proteotypic and or genotypic identification methods will more accurately yield a species level identification.
- Routine Environmental Monitoring (EM) is critical for detecting the changing trends in microbial contamination and implementation of effective control measures.

5 IMPACT ON REGULATORY UPDATES



6 REFERENCES

¹ Sandle, Tim. EU GMP Annex 1: Manufacture of Sterile Medicinal Products. European Commission: Brussels, Belgium (2022).
² EudraLex - Volume 4. Public Health, 2022, health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en.
³ Parte, A.C., Sardà Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C. and Göker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. International Journal of Systematic and Evolutionary Microbiology, 70, 5607-5612; DOI: 10.1099/ijsem.0.004332