

Abstract

To identify bacteria and fungi, pharmaceutical manufacturers rely on curated, compliant and up-to-date libraries. Results of using a proprietary library, based on over 300,000 unknown organisms recovered from environmental monitoring programs, show a two-fold reduction in unidentified strains when compared to a commercially available library. The relevance of the included library entries that represent what actually is encountered in the pharmaceutical manufacturing environment is discussed. More than just the most frequently occurring organisms must be included to provide significant library coverage for accurate identifications. Regular library updates encompassing taxonomic changes and novel organisms are imperative for superior performance, continued reliability and relevance. The library update process and its impact on results are also discussed.

Introduction

With frequent updates of proprietary bacterial and fungal libraries utilized for analysis of unknown organisms isolated from pharmaceutical manufacturing facilities, the occurrence of No Match and Genus level identifications decreases significantly over time. The addition of novel organisms, as well as updates in taxonomic changes to proprietary libraries, also increases the probability of a Species level of identification.

Continuous maintenance requires timely evaluation of sequences that are not identified to the Species level. Investigating groups or clusters of sequences that do not provide a Species level of identification is essential to building a library that contains relevant organisms with a broad range of coverage.

Sequences of type strains are derived either from a culture collection strain or by downloading the most recent type strain sequences from public databases that are published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM) and other peer reviewed journals.

Commercial libraries do not provide as high a rate of Species level identifications when compared to a proprietary library developed from organisms observed by the industries it serves. Inclusion of common or frequently occurring organisms, as well as those only observed occasionally contributes to the library's overall performance.

Discussion

When evaluating the commercially-available MicroSEQ® Fungal Library V. 2.0, it is clear that the absence of common type strains may provide inconclusive results for the end user. Figures 1A and 1B demonstrate this. The unknown organism, C305304, when compared to a continually updated library of fungal strains (Accugenix Fungal Library 28Apr08), links directly to its closest match, *Aspergillus sydowii*. Figure 1B shows the same unknown, C305304, linking to 2 strains of *Aspergillus versicolor*. In a commercial library, with a difference of only 0.09% to *Aspergillus versicolor* (its closest match), the end user is likely to misidentify this organism. A fungal library that is continuously curated and updated through observing groups of unknown organisms and adding known type strains, rather than multiple strains of the same species would be expected to provide more accurate results.

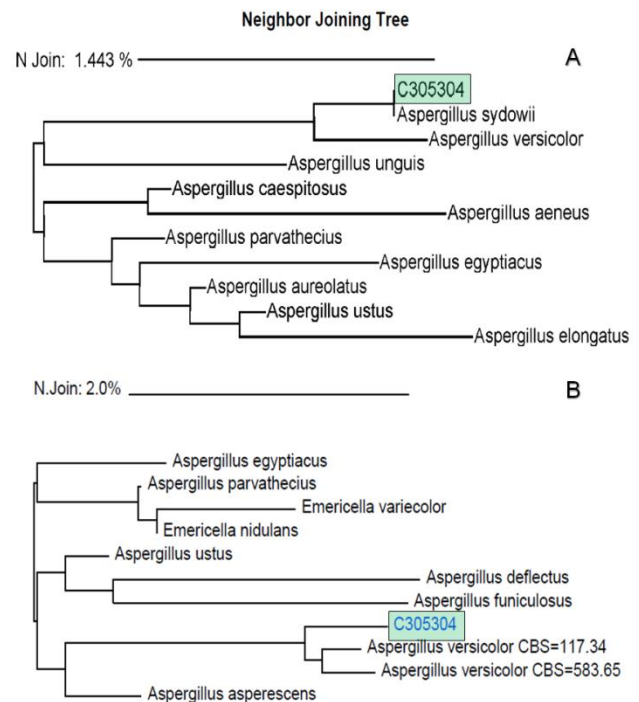


Figure 1. Phylogenetic tree used for result interpretation
A. Closest match to *Aspergillus sydowii* (0.00% difference) (Accugenix Fungal Library 28Apr08)
B. Closest match to *Aspergillus versicolor* (0.09% difference) (MicroSeq Fungal Library V. 2.0)

The current Accugenix Bacterial Library (04Sep08), containing Genera with many representative type strains will perform more effectively than one that is commercially available (MicroSEQ Bacterial Library V. 2.1). Figures 2A and 2B illustrate this with an example of an unknown *Bacillus* sp., C312683. *Bacillus marisflavi* matches exactly (0.00% difference) with the unknown organism in Figure 2A and is a clear Species level identification. The closest match, *Bacillus oleronius*, is 5.2% different from the unknown, C312683, and the phylogenetic tree suggests this is a Genus level identification. The MicroSEQ® library simply does not contain *Bacillus marisflavi*.

Library updates, to remain current, require constant maintenance and evaluation of clusters and groups of unidentified organisms. Although many strains evaluated during this process are not the most frequently occurring organisms, they provide a broad range of coverage for unknown organisms derived from the industries served. The tables in Figures 3 and 4 present a side-by-side comparison of missing species in the MicroSEQ® Libraries. The effectiveness of a library depends on its ability to identify all organisms encountered. A comparison to the most recent release of a commercially available bacterial library demonstrated that it is missing 193 entries that were closest matches for samples tested with the proprietary library. This accounted for 12% of Species level identifications. Figure 5 shows an approximation of the current performance expected for MicroSEQ® Bacterial Library V. 2.1. A similar comparison to the MicroSEQ® Fungal Library V. 2.0 revealed 136 missing organisms, accounting for more than 19% of Species level identifications (Figure 6).

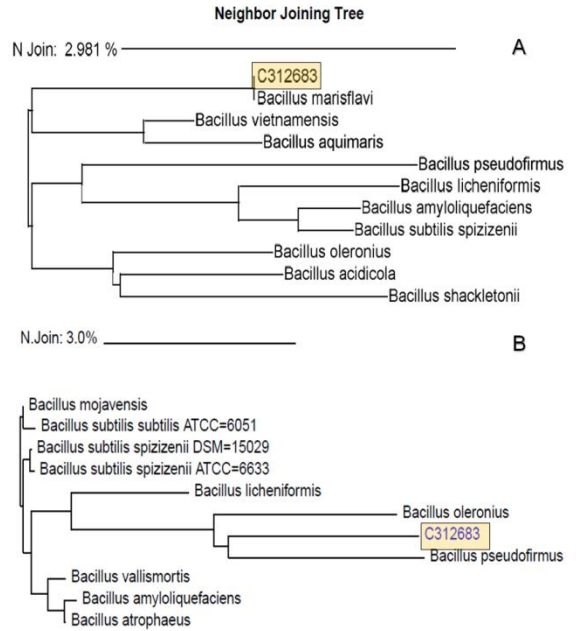


Figure 2. Phylogenetic tree used for result interpretation
 A. Closest match to *Bacillus marisflavi* (0.00% difference) (Accugenix Bacterial Library 04Sep08)
 B. Closest match to *Bacillus oleronius* (5.2% difference) (MicroSeq Bacterial Library V. 2.1)

Comparing Proprietary versus Commercial Library Entries

Reference Library 28Apr08	Alternative Library 2.0
<i>Aspergillus bisporus</i>	
<i>Aspergillus brunneo-uniseriatus</i>	
<i>Aspergillus caelatus</i>	
<i>Aspergillus caesiellus</i>	<i>Aspergillus caesiellus</i>
<i>Aspergillus caespitosus</i>	
<i>Aspergillus candidus</i>	<i>Aspergillus candidus</i>
<i>Aspergillus carbonarius</i>	<i>Aspergillus carbonarius</i>
<i>Aspergillus carneus</i>	<i>Aspergillus carneus</i>
<i>Aspergillus cervinus</i>	
<i>Aspergillus clavatoflavus</i>	
<i>Aspergillus clavatonanicus</i>	
<i>Aspergillus clavatus</i>	
<i>Aspergillus conjunctus</i>	
<i>Aspergillus crustosus</i>	
<i>Aspergillus crystallinus</i>	
<i>Aspergillus deflectus</i>	<i>Aspergillus deflectus</i>
<i>Aspergillus eburneoocreumus</i>	
<i>Aspergillus egyptiacus</i>	<i>Aspergillus egyptiacus</i>
<i>Aspergillus elongatus</i>	
<i>Aspergillus fischeri</i>	
<i>Aspergillus flavipes</i>	
<i>Aspergillus flavus</i>	<i>Aspergillus flavus oryzae</i>

Figure 3. Accugenix Fungal Library 28Apr08 vs. MicroSeq Fungal Library V. 2.0

Reference Library 04Sep08	Alternative Library 2.1
<i>Bacillus fordii</i>	
<i>Bacillus fortis</i>	
<i>Bacillus fumaroli</i>	
<i>Bacillus funiculus</i>	<i>Bacillus funiculus</i>
<i>Bacillus galactosidilyticus</i>	
<i>Bacillus gelatini</i>	
<i>Bacillus gibsonii</i>	<i>Bacillus gibsonii</i>
<i>Bacillus ginsengihumi</i>	
<i>Bacillus halmapalus</i>	<i>Bacillus halmapalus</i>
<i>Bacillus halodurans</i>	<i>Bacillus halodurans</i>
<i>Bacillus halophilus</i>	<i>Bacillus halophilus</i>
<i>Bacillus hemicellulosilyticus</i>	
<i>Bacillus herbersteinensis</i>	
<i>Bacillus horikoshii</i>	<i>Bacillus horikoshii</i>
<i>Bacillus horti</i>	<i>Bacillus horti</i>
<i>Bacillus humi</i>	
<i>Bacillus hwajinpoensis</i>	
<i>Bacillus idriensis</i>	
<i>Bacillus indicus</i>	
<i>Bacillus infantis</i>	
<i>Bacillus infernus</i>	
<i>Bacillus insolitus</i>	<i>Bacillus insolitus</i>

Figure 4. Accugenix Bacterial Library 04Sep08 vs. MicroSeq Bacterial Library V. 2.1

Conclusion

The effectiveness of the Accugenix bacterial and fungal library updates is evaluated over time. Looking at trends in data after the release of new libraries (Figures 5 and 6) provides very clear evidence that timely updates to libraries greatly increase the probability of a Species level identification. With this increase, the number of organisms that are not identified to the Species level decreases significantly. Updates to the Accugenix bacterial library have reduced the number of No Match results to less than one-half percent since 2006.

It is clear that, in order to maintain a library that has the ability to identify the majority of unknown organisms to the Species level, constant review of unidentifiable clusters or groups of organisms, whether commonly occurring or rarely occurring is imperative. The absence of frequently occurring organisms will reduce the chances of Species level identifications, as described above. As demonstrated in Figures 1 and 2, the exclusion of type strains that are less common, coupled with the inclusion of more than one strain for some entries, will result in incorrect or less specific identifications.

Bacterial Library Performance

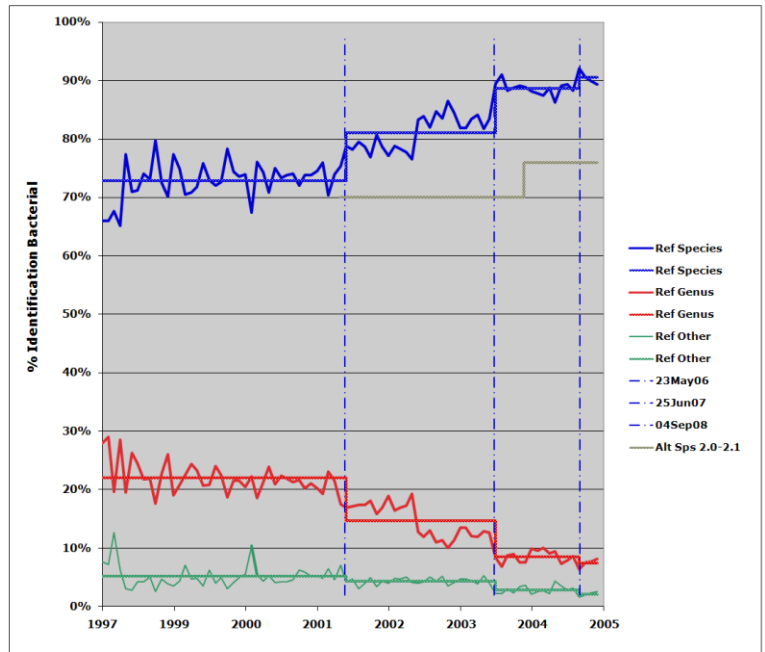


Figure 5. The effect of Bacterial Library Releases on database performance

Fungal Library Performance

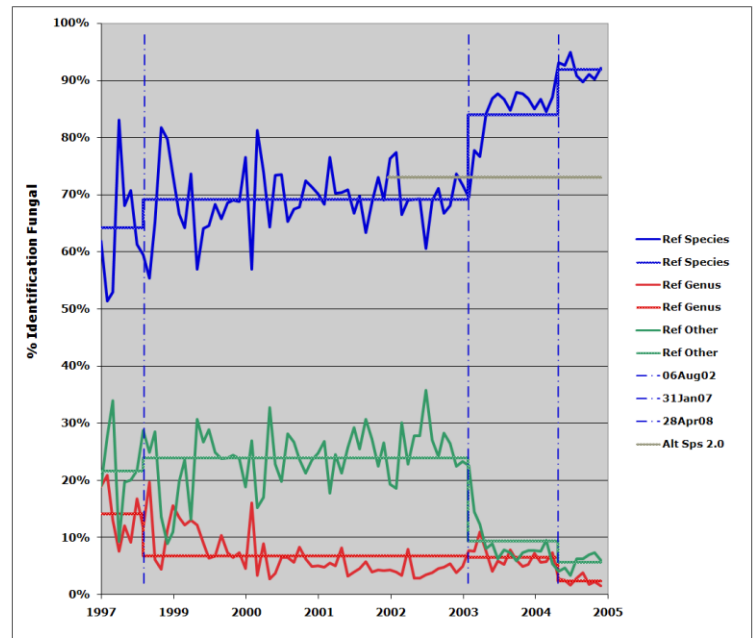


Figure 6. The effect of Fungal Library Releases on database performance

About Us

Accugenix, Inc. provides leading-edge technology in microbial identification and characterization services. Our FDA-registered lab is cGMP compliant and maintains rigorous standards competitive at the global level. We specialize in testing, analyzing and interpreting data from environmental isolates commonly found in pharmaceutical, biotechnology, medical device, nutraceutical, personal care and other manufacturing industries.

For more than 20 years, Accugenix has provided the fastest, most accurate and reliable microbial identification services to over 400 facilities around the world. Accugenix updates its validated, proprietary DNA sequence libraries annually to reflect current taxonomy and newly described relevant species. We have the industry's first Fungal Library based on the ITS region. Since inception, we have tested more than 400,000 microorganisms – more than any other service laboratory in the industry, while maintaining an on-time delivery of over 99%.

History of Accugenix

1990.

Accugenix, Inc. began as Acculab, Inc., a reference laboratory specializing in microbial identification for industry and research clients. At the time we were one of only a few service laboratories in the world offering cellular fatty acid analysis, beginning a tradition of bringing cutting-edge microbiology methods to full commercial potential and utilization.

1999.

To reflect the addition of comparative DNA sequencing to our menu of validated methods, we created Accugenix, A Division of Acculab, Inc. Since then we have sequenced hundreds of thousands of environmental isolates from over 1000 pharmaceutical and biotechnology production facilities around the world, allowing us to build the largest and most unique industry database for bacteria and fungi that often occur in clean room manufacturing environments.

2005.

Our official name changed to Accugenix, Inc. on February 25, 2005.

2008.

Accugenix GmbH, our European subsidiary, was launched in Spring 2008.

Today.

Dedicated to being the industry leader for providing the most progressive microbiology methods available, Accugenix has invested in the technology, instrumentation and expertise to conquer genetic-based testing methods, their process validation, cGMP compliance, and other rigorous regulatory standards at the global level. Accugenix continues to staff its ranks with scientists and experts to guide and/or fast-forward your transition to genotypic microbial identification.



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