applied biosystems® by life technologies™

Genotypic approach to microbial identifications outperforms phenotypic approach

Genotypic approach remains the gold standard in pharmaceutical manufacturing environment

Introduction

Accurate identification of environmental isolates is required to support active environmental monitoring programs within pharmaceutical manufacturing environments and to implement accurate Corrective and Preventive Actions (CAPAs) for products and processes to reduce the risk of contamination. The comparative gene sequence analysis of ribosomal DNA (rDNA) has been shown to have the highest accuracy of microbial identification system technologies and has been considered the gold standard for microbial identification for over a decade. Over the past several years, many of the top pharmaceutical companies worldwide have adopted the MicroSEQ® ID Rapid Microbial Identification System, which is based on comparative sequence analysis of the 16S rDNA of bacteria and 25S rDNA of fungi.

In addition to DNA sequencing, there are many phenotypic methods for identification available, such as MALDI-TOF MS.* Independent studies that compare the accuracy of the MALDI-TOF method to 16S rDNA sequencing for microorganism identification have been lacking. This paper highlights a study performed by two independent laboratories to evaluate the accuracy, repeatability, and database quality of the MALDI-TOF MS Biotyper system compared to the MicroSEQ® ID Rapid Microbial Identification System.

Accuracy

The overall accuracy of the MALDI-TOF system as determined in this study was 58%, compared to 95% for the MicroSEQ® ID system, when both culture collection strains and environmental isolates were counted together. Here, accuracy is defined as the ability to correctly identify isolates with species-level specificity. When the data are separated into two classes, the main reason for the lower accuracy in the MALDI-TOF system becomes clear. The accuracy of the MALDI-TOF system with environmental isolates collected from pharmaceutical manufacturing environments is 51%, compared to 93% for the MicroSEQ® ID system; by comparison, the accuracy of the MALDI-TOF system in identifying culture collection strains to the species level is 83%, compared to 100% for the MicroSEQ® ID system (Table 1). While the MicroSEQ® ID system clearly outperforms the MALDI-TOF system in identification accuracy across sample types, the distinction between culture collection strains and environmental isolates is

- Two independent laboratories confirm that the MALDI-TOF system has significantly lower species-level identification capability compared to the MicroSEQ® ID system, specifically for pharmaceutical isolates.
- In addition to its limitations as a phenotypic method, the MALDI-TOF system also has significant repeatability issues that likely stem from instrument variation and/or environmental growth conditions.
- The database for the MALDI-TOF system is strain-dependent and does not contain key strains for bacterial species commonly found in pharmaceutical manufacturing environments.
- Based on the variability and accuracy rates, the MALDI-TOF system does not meet the needs for routine identification of microbes found in pharmaceutical manufacturing environments as effectively as the MircoSEQ® ID system does.

*MALDI-TOF MS detects the mass profile of expressed proteins in the bacteria or fungi. The mass profile is compared to a database of previously collected species profiles (or spectra), and identification is made based on the similarity between the unknown and database spectra.

important because this implies that the **MALDI-TOF system will have significantly lower performance in a real setting vs. in testing culture collection strains.**

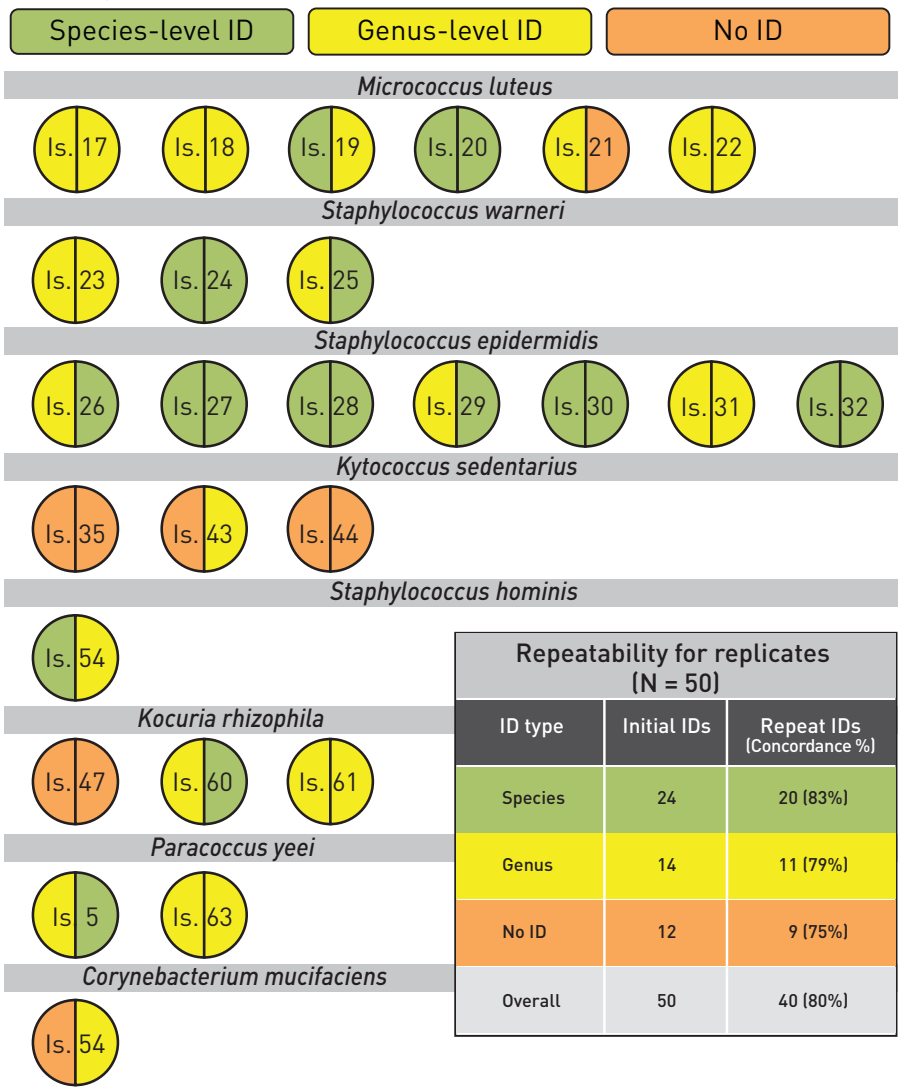
Table 1. Accuracy of identification by the MicroSEQ® ID and MALDI-TOF systems.

	Total strains (N = 77)		Environmental isolates (N = 59)		Culture collection strains (N = 18)	
	MALDI-TOF system	MicroSEQ® ID system	MALDI-TOF system	MicroSEQ® ID system	MALDI-TOF system	MicroSEQ® ID system
Species	45 (58%)	73 (95%)	30 (51%)	55 (93%)	15 (83%)	18 (100%)
Genus	19 (25%)	4 (5%)	17 (29%)	4 (7%)	2 (11%)	0 (0%)
No ID	13 (17%)	0 (0%)	12 (20%)	0 (0%)	1 (6%)	0 (0%)

Consistency

In addition to accuracy, repeatability is vital to an identification system. A subset of 50 of the environmental isolates was analyzed in duplicate to evaluate the repeatability of the MALDI-TOF system. Replicates consisted of 2 colonies from the same culture plate, harvested at the same time and run at the same time on the MALDI-TOF system. The MALDI-TOF analysis showed repeatability of 80% for all identifications, meaning that 20% of the time, the same isolate run twice on the same MALDI-TOF system plate gave a different specificity (Figure 1).

Figure 1. Two replicates per isolate (Is.) were analyzed in parallel using the MALDI-TOF system. Each half-circle represents one replicate. Circles show examples of results. Inset table shows a summary of data for all 50 isolates used in this analysis.



Repeatability for replicates (N = 50)		
ID type	Initial IDs	Repeat IDs (Concordance %)
Species	24	20 (83%)
Genus	14	11 (79%)
No ID	12	9 (75%)
Overall	50	40 (80%)

Table 2. Accuracy of the MALDI-TOF system amongst different isolates that were identified as the same species (species groups = 11, isolates = 54).

	MALDI-TOF system	MicroSEQ® ID system
Species	52%	100%
Genus	35%	0%
No ID	13%	0%

There was also variation in the identifications of the same species isolated from different environments (Table 2). The within-species accuracy rate for all species collected with multiple isolates was 52% for the MALDI-TOF system, compared to 100% for the MicroSEQ® ID system. Therefore, the same species isolated from different environments was only identifiable ~50% of the time with the MALDI-TOF system. Known sources of variation for the differences between two spectra may include strain-to-strain differences or instrument-related variation in the spectra, which would cause significant issues related to the expected accuracy and validation of the MALDI-TOF system.

Impact of database quality on accuracy and repeatability

The accuracy difference between culture collection isolates and pharmaceutical isolates highlights a main difference between the MALDI-TOF

Figure 2. Comparison of a subset of culture collection and environmental isolate identifications with the MALDI-TOF and MicroSEQ® ID systems (N = 26).

system and the MicroSEQ® ID system. The MALDI-TOF system identifications are dependent upon the protein expression of the microorganisms, and the protein expression profiles will vary depending on environmental conditions and small genetic differences between strain types. However, the 16S rDNA sequence used for the MicroSEQ® ID system is conserved across all bacterial strains and is not affected by environmental conditions. This means that the MicroSEQ® ID system will produce consistent results regardless of the isolate's origin. The MALDI-TOF system relies on an aspect of the organism that is subject to environmentally induced variation, making identification of organisms in the pharmaceutical manufacturing environment less reliable using that system.

Additional investment is needed to determine repeatable culture conditions that will give a reliable level of reproducibility. Because of the susceptibility to strain and environmental variation, any MALDI-TOF MS database will be only as good as the samples that are included in it. Evidence of this can be seen in this study by comparing results from the analysis of culture collection strains to the same species collected as environmental isolates. For example, three strains of *Staphylococcus warneri*, a species commonly found in the pharmaceutical manufacturing environment, could be identified by the MALDI-TOF system only to the level of the genus *Staphylococcus*, but were identified to the species level as *S. warneri* using the MicroSEQ® ID system (Figure 2). Species-level IDs for some environmental isolates of *Bacillus subtilis*, *Micrococcus luteus*, and *Kocuria rhizophila* were also unobtainable using the MALDI-TOF system. However, the MicroSEQ® ID system identified all isolates of all species to the same level.

The accuracy of MALDI-TOF MS (or any technology that gives variable results for strains of a given species) suffers when the databases it refers to do not adequately represent the widest diversity of strains. If the diversity is caused by environmental pressures or antibiotic treatment, the task of improving the identification accuracy (by building a custom database or expanding an existing database, for example) can be quite laborious and time-consuming and may not even be possible due the variability of conditions. In contrast, the MicroSEQ® ID system is robust because environmental conditions do not affect the rDNA sequences of bacteria and fungi. Thus, a database constructed from a type strain or a well-characterized strain of interest is sufficient to enable accurate identification of isolates of a species, regardless of their growth conditions.

Conclusions

Results from this study highlight the importance of understanding a technology and how it performs in a pharmaceutical manufacturing environment when selecting a microbial identification system. It is critical to evaluate whether the collection of strains and species represented in a system's database is applicable to the environment of the intended use. Additionally, the accuracy, repeatability, and influence of growth conditions should be taken into account when implementing a microbial identification system. Each of these factors will greatly impact the feasibility, time, and total cost to implement the system, as well as the ability to support an effective environmental monitoring program, accurate tracking and trending of microorganisms, and CAPAs.

The factors contributing to low accuracy, low repeatability, and complexity with respect to database coverage and creation will greatly impact the ability to validate the MALDI-TOF method for use in a cGMP facility, because defining a true identification will be very difficult.

- With an accuracy of only 58% for the MALDI-TOF system (Table 1), a second technology would be required to identify the remaining isolates
- The additional cost associated with a second system (including system validation and operation, or outsourcing) should also be considered in the overall investment for implementing a MALDI-TOF system
- With 95% accuracy, and consistent results regardless of environmental stress and growth conditions, the MicroSEQ® ID system is better suited for microbial identification
- The accuracy and repeatability of the MicroSEQ® ID system make it easy to validate and implement for routine use in a pharmaceutical manufacturing environment while minimizing costs associated with repeat testing

Methods

A total of 18 culture collection strains from the CCUG culture collection (including 14 type strains) and 59 isolates from a pharmaceutical manufacturing environmental monitoring program were analyzed using both the Bruker MALDI-TOF MS system and the MicroSEQ® ID Rapid Microbial Identification System. Identification testing was performed by two independent laboratories. Assay protocols were followed as recommended by the system manufacturer for both methods. Spectral values for MALDI-TOF system analysis were ≥2.0 for species identification and ≥1.7 for genus identification. For the MicroSEQ® ID system, species identification was determined using a cutoff of ≥98.5% for species and ≥95% for genus. A list of genera represented by this study is shown in Figure 3.

Figure 3. Samples tested in this study included species from these genera.

Aspergillus	*Micrococcus*
Bacillus	*Paracoccus*
Burkholderia	*Pseudomonas*
Candida	*Ralstonia*
Corynebacterium	*Roseomonas*
Geobacillus	*Serratia*
Klebsiella	*Sphingomonas*
Kocuria	*Staphylococcus*
Kytococcus	*Stenotrophomonas*

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

© 2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.
Printed in the USA. **CO24216 0112**