Enumeration and species level identification of complex mixtures using colony mass spectrometry

Rosa Sava, Michael Coryell Ph. D, Paul Carlson Ph. D

Laboratory of Mucosal Pathogens and Cellular Immunology, Division of Bacterial Parasitic and Allergenic products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, FDA

Abstract

Live biotherapeutic products (LBPs) contain live organisms, such as bacteria, and are developed for treatment, prevention, or cure of conditions that have a strong link to the host's microbiome. In the United States, LBPs are regulated as a drug product. As such, they require testing for identity, purity, and potency before release. Because the therapeutic effects of LBPs rely on living organisms and the mechanisms of action are generally undefined, potency assays must provide accurate and reproducible measurements of viable organisms in a product, generally a measurement of the total colony forming units (CFU) per dose of product in its final form. However, potential LBPs often consist of two of more bacterial strains, sometimes of closely related organisms with similar growth requirements, complicating the process of CFU enumeration and identity testing. Colony mass spectrometry is commonly used for rapid identification of bacteria and fungi cultured from clinical specimens and is sensitive enough to distinguish strain-level identities for many organisms. The purpose of this research project is to develop and validate a method for simultaneous enumeration and identification of multiple bacterial strains in a complex mixture using a combined approach of dilution plating for CFU counts and colony mass spectrometry for identification of individual colonies.

Introduction

- LBPs are defined as a biological product that contains live organisms, is applicable to the prevention, treatment, or cure of a disease or conditions that affects human beings and is not a vaccine.
- Since LBPs are regulated as a drug in the United States, thorough testing must be done to ensure product safety and efficacy. Products containing live organisms rely on potency assays to confirm identity and viability of those organisms, typically as the total colony forming units (CFU) per dose.
- LBPs may contain multiple organisms that grow on similar substrates and media types, requiring testing method which accurately show all organisms present in the LBP.
- Colony mass spectrometry (colony MS) can rapidly identify microorganisms down to the species level with low costs per sample.
- Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry builds a peptide mass fingerprint (PMF) based on the mass-to-charge ratio of ions from the sample. The PMF is then compared to reference spectra of known microorganisms to determine the identification of the sample.

Results

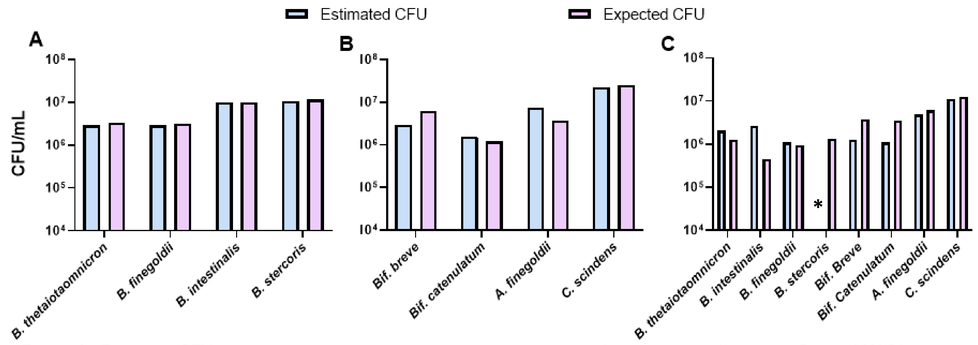


Figure 1. Estimated CFU values were determined by the relative abundance of colonies identified by the Bruker MALDI Biotyper while expected CFU values were determined using direct plate counts. Colonies were counted and selected for colony MS from YCFA agar plates inoculated with cultures grow in YCFA broth and serially diluted in phosphate buffered saline (PBS). **A)** Comparison of CFU/mL values from *Bacteroides* species. **B)** Comparison of CFU/mL values from *Bifidobacterium*, *Alistipes*, and *Clostridium* species. **C)** Comparison of CFU/mL values from an 8-strain mixture.
**An estimated CFU/mL value for B. stercoris was not able to be determined because no colonies from the mixture were identified as B. stercoris by colony MS.*

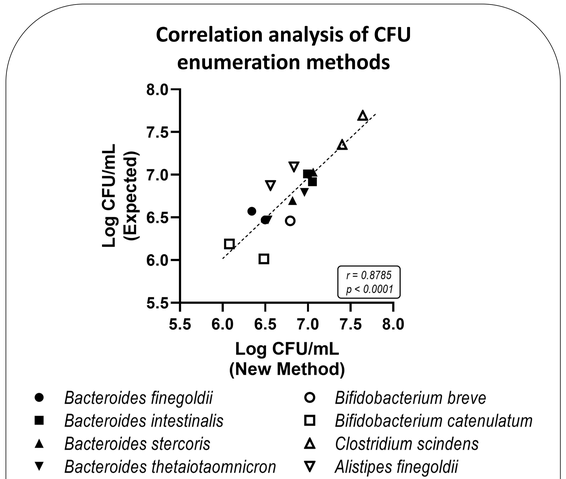


Figure 2. Log-transformed CFU/mL values determined for bacterial mixtures of up to four bacterial strain using this method strongly correlate with expected values based on conventional serial dilution plate counts of pure cultures prior to mixing (Pearson-r = 0.8785, $p < 0.0001$).

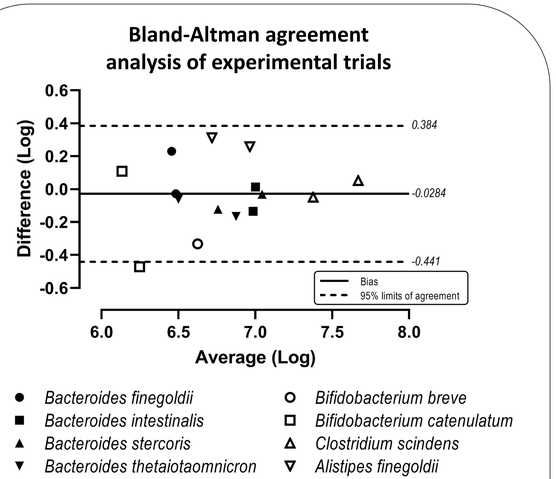
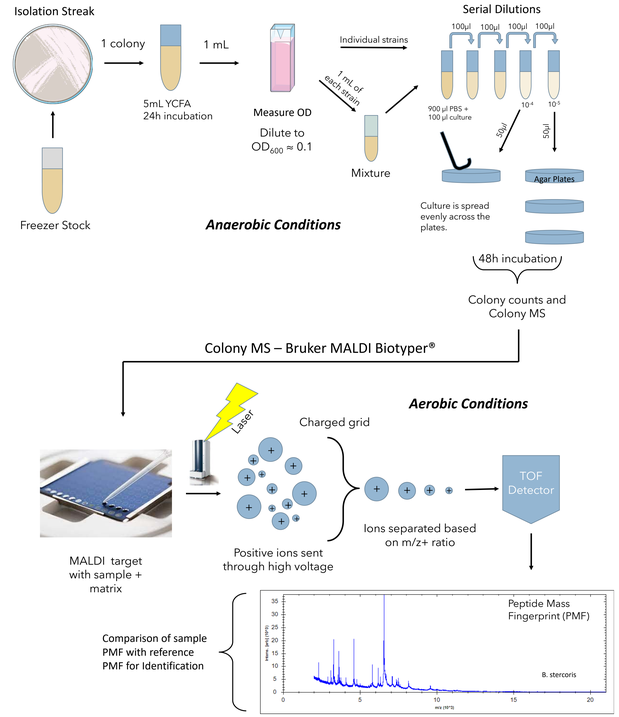


Figure 3. The mean difference (bias) between methods of log transformed CFU/mL values was -0.0284 ± 0.413 (95% CI), giving 95% limits of agreement from -0.441 to 0.384 (represented by dashed lines). A bias value near 0 indicates little systematic difference between the methods while limits of agreement provide an estimate of random error.

Materials and Methods



Conclusion

- This method shows promise as a way for simultaneous enumeration and identity confirmation of defined bacterial mixtures.
- Differences between estimated and expected CFU values could arise from inadequate sampling depth or biases in colony selection and/or exclusion criteria. Care should be taken to understand and minimize and such biases.
- More replicate trials will be performed in order to further characterize the strengths and limitations of this method.
- Future experiments with more complex mixtures will be beneficial to see the feasibility of this method for use in manufacturing and testing of LBPs.

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