B109 Evaluation of Alternative Methods to 16S rRNA Gene Sequence Analysis for Microbial Forensic Applications

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After attending this presentation, attendees will have learned about broad-range PCR-based assays for improved identification of bacterial species, how additional bacterial gene targets can be used to perform sequence analysis where other methods are insufficient, and how this type of assay can be applied in a variety of fields, including microbial forensics.

This presentation will impact the forensic community and/or humanity by demonstrating how more discriminating techniques for bacterial identification and additional gene sequence information allow more definitive information to be obtained and used for source attribution during investigation of an outbreak, biocrime, or bioterrorism event. These also provide a strong foundation for the development of future methods of strain typing or fingerprinting similar to human forensic STR analysis.

In the wake of the 2001 anthrax letter attack, the field of microbial forensics has been propelled into the spotlight. Increasing interest in biodefense and clinical microbiology applications for current outbreak, bioterrorism and biocrime analysis has necessitated method improvement. These have centered on identification and differentiation of pathogens to species and even strain levels. As an emerging field, much work is needed to prepare microbial forensics for the scrutiny of current forensic standards. One area of improvement involves the development of more discriminating identification methods, with the ultimate goal of source attribution. Inherent variation and short generation times result in nucleotide differences among bacterial gene sequences, complicating the process of matching species or strains. These discrepancies, often understood and accepted within the microbiology field, pose a challenge when considered from the legal aspect of microbial forensics. Inherent uncertainties are difficult to explain to judges and juries as convincing evidence. Methods utilized in a microbial forensic investigation must also withstand *Daubert* and *Frye* tests for admissibility as valid evidence attributed to a biocrime or act of bioterrorism. This requires continued research to improve identification methods to ensure microbial forensic techniques are comparable to the current stringent regulations for DNA-based tests for human identification. Recent molecular sequencing work performed in bacteriology and molecular development labs is an important starting point for continued work to prepare microbial forensics for scrutiny by the forensic and legal communities. Improved methods are also necessary to facilitate diagnosis and treatment of these bacteria in clinical microbiology and public health settings.

Broad-range PCR methods are useful for bacterial identification as culturing some species is extremely slow, time-consuming, or even impossible in the lab. Inexact biochemical analyses are not always specific enough to make a confident identification and comparison based upon sequencing of the *16S rRNA* gene for many species. *Streptococcus* and *Bacillus* species include human bacterial pathogens that cannot be completely and efficiently identified by these current available diagnostics. At the New York State Department of Health's Wadsworth Center, many clinical isolates of these species analyzed by the traditional *16S rRNA* gene sequence analysis demonstrate significant nucleotide sequence similarity within this gene. Additional broad-range PCR assays are needed to allow further discrimination of these species as agents of nosocomial, community, or food borne outbreaks, or even bioterrorism.

This study, performed at the Wadsworth Center, evaluated alternative gene targets *cpn60, tuf,* and *rpoB,* in broad-range PCR-based assays with *Streptococcus* and *Bacillus* for nucleotide sequence variation among species. Gene sequences with greater variation will allow improved discrimination and identification of the clinical isolates of these species received by this reference laboratory. Both published and novel primers were used for amplification of the target genes. Sequence analysis of these amplicons demonstrated an increase in nucleotide variation among species compared to their *16S rRNA* gene sequences. A preliminary blinded assay exhibited successful identification of *Streptococcus* and *Bacillus* species tested with primer sets for these target genes. Continued work including optimization and blinded validation analysis must be performed with the primer sets in progress for these species.

The design of improved methods for conclusive identification and classification of bacterial pathogens is a crucial step towards confident attribution of a species or strain as the source of any type of biological incident. The creation of databases containing sequence information obtained through this work will be implemented for future use at the Wadsworth Center. These databases will assist in the development of improved techniques for both clinical microbiology and microbial forensic applications. Obtaining additional gene sequence information provides a foundation for future methods of strain typing or fingerprinting more similar to human forensic STR analysis. Collaboration for continued advances with these assays will ensure the fields of epidemiology, microbiology, and microbial forensics are prepared to meet the challenges facing investigation and source attribution of biological events.

Microbial Forensics, Broad-Range PCR, Gene Sequence Analysis

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