B93 Rapid Genotyping of a Global Collection of Bacillus Anthracis Isolates Using Pyrosequencing

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After attending this presentation, attendees will have a greater understanding of the genetic diversity present among Bacillus anthracis (BA)—the causative agent of anthrax, the technology used to obtain genotyping results, and how such information can be used to aid investigations of bio-crimes.

The proposition underlying the work is that new technologies such as Pyrosequencing will impact the forensic science community by allowing rapid genotyping using a greater variety of sample types and with greater ease than traditional methods.

The 2001 anthrax attacks using the U.S. postal system have led to the development of an emerging discipline: microbial forensics. Broadly, this field is dedicated to the use of science to solve crimes using biological agents. One of the potentially most powerful pieces of information is genetic material obtained from the organism used in an attack. Strains or isolates of bacteria and viruses often may be distinguished from one another and assigned to a genotype based on sequence variations. Matching genotypes of material used in an attack with possible natural or laboratory sources may provide investigative leads.

Traditionally, genotyping of *Bacillus anthracis* has used DNA fingerprinting methods such as AFLP (Amplified Fragment Length Polymorphism PCR) and MLVA (Multi Locus VNTR Analysis). These powerful techniques allow strain- and sub-strain-level identification. However, these methods are technically challenging and not suitable for mixed environmental samples, especially if there are no viable isolates. An additional method of genotyping is based on sequencing of the protective antigen (*pag*A) toxin gene. Using standard sequencing methods, Paul Keim's lab discovered several single nucleotide polymorphisms (SNPs) that allowed BA stains from around the world to be categorized into six *pag*A genotypes (I-VI).

Pyrosequencing is an alternative technology for obtaining genetic information based on sequencing by synthesis. Using the published SNPs present in the *pag*A gene, pyrosequencing assays were designed to allow genotyping of BA. The authors are currently genotyping a global collection of BA isolates, with 118 isolates from 35 countries and 18 human and animal hosts. To date, the most commonly observed genotypes have been Types I and V, which respectively represent the Sterne-Ames and Western North American diversity groups. Additionally, genotyping was performed directly on soil samples associated with anthrax outbreaks in cattle in Texas and South Dakota. Genotyping can be obtained within 3.5 hours of receipt of either environmental samples or pure isolates and requires only basic molecular biology laboratory skills. In conclusion, pyrosequencing is a rapid, simple, cost-effective means for performing high throughput genotyping with a wide variety of sample types.

Microbial Forensics, Bacillus Anthracis, Genotyping

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