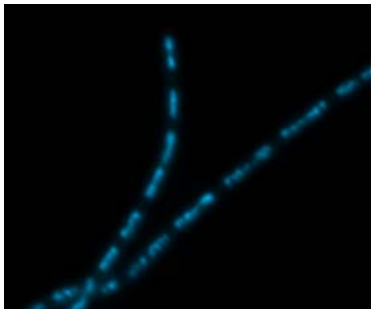
LOS ALAMOS TECHNOLOGIES FOR *BACILLUS ANTHRACIS* IDENTIFICATION



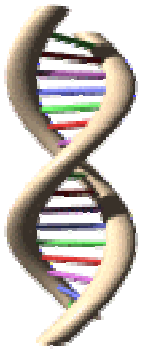
At Los Alamos National Laboratory, a United States Department of Energy/National Nuclear Security Administration facility, intensive research on *B. anthracis* over the past several years has led to a wealth of information on the *B. anthracis* genome sequence and elite technologies for detecting and identifying the organism down to its precise DNA fingerprint.

Los Alamos technologies have been applied both in the field and in the laboratory, and some have already been transferred to agencies with national responsibilities for investigating and resolving anthrax diagnoses. Specialty analysis of the DNA sequence can reveal similarities or differences among the *B. anthracis* found in various cases of infection. The degree of relatedness among different samples can also be determined, in much the same way that human DNA fingerprinting is used to establish family relationships.

Genetic Analysis

Information gleaned from the study of the *B. anthracis* genome has resulted in Los Alamos' development of unique reagents for specifically amplifying fragments of the *B. anthracis* genome using PCR (polymerase chain reaction). These reagents have been made available to federal agencies and can be used for the rapid detection of *B. anthracis* in times of concern. Some of these reagents were applied in the investigation of the causative strains of *B. anthracis*, such as an outbreak in 1979 led to the death of many people in the Former Soviet Union, in the worst human anthrax outbreak in recent history.

Los Alamos has also developed several new specialty genetic analysis tools for *B. anthracis* identification. Because these technologies are so new, they have not yet been transferred to the responding agencies.



The most mature of these technologies -- called Amplified Fragment Length Polymorphism analysis (AFLP) -- has been developed and optimized over the past several years to analyze *B. anthracis* samples from naturally occurring anthrax outbreaks around the world. In AFLP, scientists extract DNA from the bacteria and “cut” it into a specific set of small fragments using enzymes that recognize specific stretches of DNA in the genome. From these small DNA fragments, a much-reduced subset is then amplified (duplicated over and over) by PCR. The lengths of the PCR products are analyzed and compiled into a “fingerprint” that is added to a database where it can be read and interpreted by comparison to others in the database.

An extensive collection of *B. anthracis* AFLP fingerprints has been assembled and is used as a resource for matching or relating an unknown sample to the large number of fingerprints in the collection.

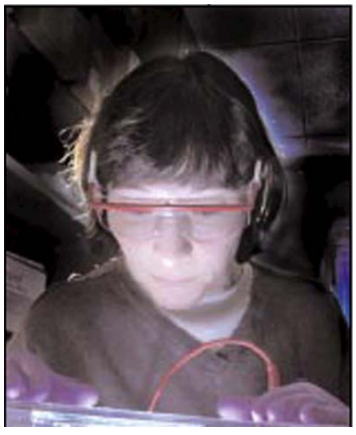
In another recent investigation the AFLP test was used to reveal important details of the *B. anthracis* used in the Iraqi biological weapons program in samples obtained during the UNSCOM inspections.

A newer technique for genetic analysis of *B. anthracis* is Multiple Locus Variable Number of Tandem Repeat analysis (MLVA), which gives a very high-resolution DNA fingerprint. MLVA recognizes the presence of repeated DNA sequences in the genome, using a set of specific "markers" that surround these repeats. Some of the markers in use were originally identified in AFLP profiles. MLVA is somewhat more sensitive than other fingerprinting methods to slight genetic variations among different strains of *B. anthracis*. A large database of MLVA fingerprints is also available. This method is in use at Los Alamos and new MLVA markers are being developed in the laboratory of a longtime Laboratory collaborator, Dr. Paul Keim, at Northern Arizona University. Analysis of an unknown *B. anthracis* strain using the combined approaches of AFLP and MLVA analysis produces a very specific genetic signature.

HOW WE'VE USED THE TECHNOLOGY

An example of the kind of forensic work involving the Los Alamos AFLP fingerprint collection is the case of an anthrax outbreak that occurred in central Australia in 1994. Los Alamos was able to determine that the disease was due to a *B. anthracis* strain that came to Australia from cattle imported during the 1850s from India. The infected animals were buried and the outbreak occurred when the buried carcasses were disturbed more than a century later. The spores were inhaled by grazing cattle, which in turn developed anthrax from these 144-year-old spores.

To obtain an even closer look at genetic differences among *B. anthracis* strains, an even newer molecular technology is used. Single Nucleotide Polymorphism (SNP) analysis uses a flexible, microsphere array that is analyzed on a flow cytometer platform. Los Alamos scientists have developed SNP analysis assays to target potential antibiotic-resistance genes, toxin genes, and sites in the *B. anthracis* genome that may have undergone deliberate genetic modification or "engineering." These ongoing research activities are part of our continuing effort to understand the *B. anthracis* genome and apply this knowledge to biological threat reduction needs.



Instrumentation

An emerging technology at Los Alamos for bacterial pathogen analysis is an ultrasensitive flow cytometer that measures the size of single DNA fragments in a DNA fingerprint. This benchtop instrument was developed to replace more cumbersome pulsed-field gel electrophoresis equipment used today in most public health labs for tracking foodborne disease outbreaks. The DNA fragment sizing

flow cytometer is 100 times faster and 200,000 times more sensitive at analyzing DNA samples than conventional gel electrophoresis. It requires less than two-trillionths of a gram of DNA to perform the analysis. The patent for this technology was licensed to a small company within the past year.

Prevention

Los Alamos is developing a method for neutralizing the deadly toxins released by pathogenic bacteria, such as those that cause anthrax, by using a decoy molecule –a receptor-mimicking molecule – that stops the spread of the toxin by preferentially binding the bacteria's toxin and thus keeping it from binding to immune system cells. Unlike treatments for anthrax exposure that use antibiotics, the decoy molecule is designed to be used prior to exposure to biowarfare agents like anthrax. (See news release, "Takes two to tango: Neutralization of staph toxins," May 23, 2001)

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