

## Stable-Isotope Fingerprinting System for CBW Safeguards and Forensics

### Natural, Built-in “Bar-codes”

#### DOD and Homeland Security Issues and Technology Impact

In the wake of the September 11<sup>th</sup> terrorist attacks and the subsequent incidents of “anthrax letters”, there is a growing public concern over large-scale attacks with hazardous biological and chemical weapons (anthrax, smallpox, sarin, cyanide, *etc.*). It is important to characterize terrorist attacks and materials from terrorist labs, so that the perpetrators can be apprehended, and future attacks can be intercepted and prevented. For this reason, reliable forensic methods are needed to determine the origins of biological and chemical materials used in terrorism. However, current methods for CBW identifications (chemical formula, DNA sequences of bacteria, immunoassay, culturing) are insufficient to determine the source of the materials. For example, anthrax spores collected from the attacks in the fall of 2001 were all identified as the Ames strain. However, this particular strain of anthrax spores could have been produced in several different laboratories and locations within the U.S. or abroad. Despite rapidly developing and promising technologies for DNA-sequencing and immunoassay it is still very difficult to distinguish different isolates of the same strains of pathogenic bacteria, largely because many bacteria are genetically very homogeneous. Clearly, additional methods are needed for identifying the sources of biological and chemical weapons. Naturally-occurring stable isotopes of light elements (hydrogen, carbon, nitrogen, oxygen, *etc.*) of CBW, which serve as built-in bar-codes, can be used for their batch- and isolate-level identification.

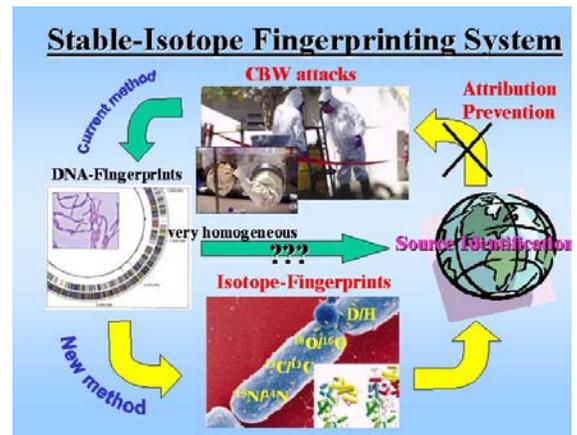
#### Technical Concept

Natural stable isotopes of carbon, hydrogen, nitrogen, oxygen and other light elements of CBW possess multiple stable isotopes (*e.g.*,  $^{13}\text{C}/^{12}\text{C}$ ,  $\text{D}(^2\text{H})/\text{H}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$ , *etc.*). The abundance of these stable isotopes varies depending on:

- Starting raw materials and substrates used.
- Manufacturing processes and culturing conditions
- Geographic regions of growth.

Variations in the abundance of stable isotopes are generally small, but can be readily detected with modern techniques of mass spectrometry.

Recent applications include the identification of geographic regions for agricultural products (cocaine, alcohol, *etc.*) and the detection of counterfeited pharmaceutical products. It is also known that chemical reagents (PCBs, benzene, *etc.*) from various manufacturers have distinct isotopic ratios. Recently, we proved the hypothesis that bacteria also possess “stable-isotope fingerprints,” depending on their growth media and culture conditions (Horita and Vass, *J. Forensic Sci.*, in press). Cultured bacteria faithfully inherited the isotopic composition of media waters and substrates in predictable manners and bacterial of the same strain, which grew in media water and substrates of different isotopic compositions, had readily distinguishable isotopic signatures.



*This innovative fingerprinting system provides batch- or isolate-level identification of CBW using naturally-occurring stable isotopes of light elements*

## Development Approach

Four areas of R&D are focused in order to develop a stable-isotope fingerprinting system of CBW for safeguard and forensic applications.

### *1. Procedures for Sample Collection and Handling*

- Size-fractionated sampling of BW from air-borne particles using advanced cascade impactors.
- Cleaning and removal of coating materials (e.g., siliconized kaolin), which are used to make “weaponized” BW agents amenable to aerosolization.
- Establishing sterilization processes (e.g.,  $\gamma$ -ray irradiation) and other handling procedures without altering the isotopic signatures of CBW.

### *2. Advanced Methods of Isotopic analysis for CBW*

- Reduction of the sample size to the sub- $\mu$ g range, because samples collected from the environment of crime scenes of bioterrorism could be very limited in amount.
- High data throughput to build a large database and to process “real-case” samples in a timely fashion
- Multiple-isotope analysis ( $^{13}\text{C}/^{12}\text{C}$ ,  $\text{D}(^2\text{H})/\text{H}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{34}\text{S}/^{32}\text{S}$ ) to increase the resolving power of the stable-isotope fingerprints
- Refractory biomolecules (e.g., lipids), if the isotopic signatures of bacterial cells alter over prolonged time

### *3. “Stable-isotope Fingerprints” of CBW*

- Establishing (“bar-coding”) stable-isotope fingerprints of CBWs currently archived and stockpiled in various federal and private chemical and biological laboratories.
- Establishing stable-isotope fingerprints of raw materials and growth media from major U.S vendors and suppliers, which can be used to produce and grow various CBW.
- Establishing the relationship of isotopic composition between raw materials/growth media and CBW under typical production-growth conditions.

### *4. Rigorous procedures for Forensic Attributions*

- Matching the stable-isotope fingerprints of CBW collected from the environment of terrorist attacks or from a suspect individual/laboratory with a database for archived and stockpiled materials
- Narrowing down manufactures and vendors, from which the raw materials/growth media were purchased, and geographic locations of the laboratory.

## ORNL Facilities and Related Programs

Oak Ridge National Laboratory has developed the Chemical Biological Mass Spectrometer Block II in a US Army program (Dr. W.H. Griest, program manager), which is aimed at providing field-deployable mass spectrometers for rapid detection and identification of CBW. We have a biolaboratory built to biosafety level 3, and have archived many isolates of gamma-killed pathogens. We are also equipped with a state-of-the-art isotope laboratory, where the isotopic fingerprints of CBW can be accurately determined.

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