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TIGER: the universal biosensor

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Abstract

In this work, we describe a strategy for the detection and characterization of microorganisms associated with a potential biological warfare attack or a natural outbreak of an emerging infectious disease. This approach, termed TIGER (Triangulation Identification for the Genetic Evaluation of Risks), relies on mass spectrometry-derived base composition signatures obtained from PCR amplification of broadly conserved regions of the microbial genome(s) in a sample. The sample can be derived from air filtration devices, clinical samples, or other sources. Core to this approach are "intelligent PCR primers" that target broadly conserved regions of microbial genomes that flank variable regions. This approach requires that high-performance mass measurements be made on PCR products in the 80–140 bp size range in a high-throughput, robust modality. As will be demonstrated, the concept is equally applicable to bacteria and viruses and could be further applied to fungi and protozoa. In addition to describing the fundamental strategy of this approach, several specific examples of TIGER are presented that illustrate the impact this approach could have on the way biological weapons attacks are detected and the way that the etiologies of infectious diseases are determined. The first example illustrates how any bacterial species might be identified, using *Bacillus anthracis* as the test agent. The second example demonstrates how DNA-genome viruses are identified using five members of *Poxviridae* family, whose members includes Variola virus, the agent responsible for smallpox. The third example demonstrates how RNA-genome viruses are identified using the *Alphaviruses* (VEE, WEE, and EEE) as representative examples. These examples illustrate how the TIGER technology can be applied to create a universal identification strategy for all pathogens, including those that infect humans, livestock, and plants.

Keywords: Pathogen detection; Microbe; Biosensor; Biodefense; Infectious disease

1. Introduction

Mass spectrometry-based methods for analysis of nucleic acids continue to mature as hardware, software, and sample purification protocols grow in sophistication. While there are a number of relatively simple methods one can use to address straightforward analytical questions related to nucleic acids (e.g. slab gels, capillary gel electrophoresis), the recent tendency is to move towards more sophisticated analytical

Abbreviations: CDC, centers for disease control; EEE, eastern equine encephalitis; ESI–MS, electrospray ionization mass spectrometry; FTICR, Fourier transform ion cyclotron resonance; PCR, polymerase chain reaction; TIGER, triangulation identification for the genetic evaluation of risks; TOF, time-of-flight; USDA, United States Department of Agriculture; UTHSC, University of Texas Health Science Center; VEE, Venezuelan equine encephalitis; WEE, western equine encephalitis; bp, base pair; BW, biological weapon; A, adenosine; G, guanosine; C, cytidine; T, thymidine; FWHM, full-width half-maximum; SNPs, single nucleotide polymorphisms

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platforms to answer more complex questions. In this work we will introduce the concept of Triangulation Identification for the Genetic Evaluation of Risks (TIGER). TIGER employs high-performance electrospray mass spectrometry (either FTICR or TOF) to derive base compositions of polymerase chain reaction (PCR) products. Core to this approach are "intelligent PCR primers" that target broadly conserved regions of microbial genomes that flank variable regions. This approach requires that high-performance mass measurements be made on PCR products in the 80–140 bp size range in a high-throughput, robust modality. As will be demonstrated, the concept is equally applicable to the detection of bacteria and viruses and could be further applied to fungi and protozoa. In addition to describing the fundamental strategy of this approach, we present several specific examples of TIGER that illustrate the potential impact this approach could have on the way we detect biological weapons attacks and the way we determine the etiology of infectious diseases.

A key problem in determining the cause of a natural infectious outbreak or a bioterrorist attack is the sheer variety of organisms that can cause human disease. According to a recent review [1], there are over 1400 organisms infectious to humans; many of these have the potential to emerge suddenly in a natural epidemic or to be used in a malicious attack by bioterrorists. This number does not include numerous strain variants, bioengineered versions, or pathogens that infect plants or animals. Paradoxically, much of the new technology being developed for detection of biological weapons incorporates a polymerase chain reaction step based upon the use of highly specific primers and probes designed to selectively detect certain pathogenic organisms. Although this approach is appropriate for the most obvious bioterrorist organisms, like smallpox and anthrax, experience has shown that it is very difficult to predict which of hundreds of possible pathogenic organisms might be employed in a terrorist attack. Likewise, naturally emerging human diseases that have caused devastating consequences to public health have come from unexpected families of bacteria, viruses, fungi, or protozoa. Plants and animals also have their natural burden of infectious disease agents and there are equally important biosafety and security concerns for agriculture.

A major conundrum for public health protection, biodefense, and agricultural safety and security is the need to be able to rapidly identify and characterize infectious agents, yet there is no existing technology with the breadth of function to meet this need. In this paper we describe broad-function technology based on mass spectrometric detection that enables the rapid, sensitive, and cost-effective identification of a broad range of infectious microorganisms, including natural human pathogens, bioterrorist agents, and agricultural pathogens. The use of such broad-function technology may be the most practical way to simultaneously survey for all forms of pathogens.

To achieve this objective, we have abandoned the notion of detecting specific target organisms on a "one off" basis, and instead have developed a strategy to identify all the organisms present in the sample without anticipating which might be present. TIGER is based upon the principle that, despite the enormous diversity of microbes, all domains of life on earth share sets of essential common features in the biomolecules encoded in their genomes. We use these common features in an identification strategy that relies on high-performance ESI–MS analysis of broad-range PCR amplification products and base-composition analysis. The base compositions from multiple primer pairs are used to "triangulate" the identity of the organisms present in the sample.

In this paper, we describe the basic principles of TIGER, and provide examples of applications of the technology in environmental surveillance for an aerosol attack with a biological weapon, or for analysis of a human clinical sample. We describe three examples in which the TIGER approach is used to detect and characterize microbes associated with a potential BW attack or naturally occurring pathogen. The first example describes how any bacterial species might be identified, using *Bacillus anthracis* as the test agent. The second example demonstrates how DNA-genome viruses are identified using five members of *Poxviridae* family, whose members includes Variola virus, the agent responsible for smallpox. The third example demonstrates how RNA-genome viruses are identified using the *Alphaviruses (*VEE, WEE, and EEE) as representative examples. These examples illustrate how the TIGER technology can be extended to create a seamless biosensor network for the universal identification of all pathogens.

2. Experimental/materials and methods

2.1. Bacterial genome isolation from air samples

Air samples were collected on a Spincon portable air sampler system, model number PAS540-10 (Camber Corporation, Huntsville, AL) or on dry filter units (DFUs). The airflow on the Spincon unit is approximately 450 L/min and the material is collected directly into a PBS/detergent liquid matrix. For the DFUs, the airflow is approximately 850 L/min and the material is collected onto a 2" polyester fiber filter. Sample times for each collection are indicated in the text. For the DFUs, filters were collected from the units and up to four filters were combined in 20 mL of phosphate buffered saline (pH 7.0) containing 0.1% Tween-20 detergent. The filters and solution were shaken by hand for 30 s prior to storage at 4 °C. Prior to genomic extraction, the filters and solution were shaken again for 30 s and the resulting solution was filtered through a 25 mm 0.2 μm Supor-200 filter (Pall Corporation, Ann Arbor, Michigan). Spincon material was filtered directly by this same method. The resulting Supor-200 filters were then subjected to bead beating by placing the filter in a 1.5 mL tube containing ∼100 μg of 0.7 mm zirconium beads (Biospec Products, Bartlesville, OK) and 350 μL of ATL buffer from Qiagen (Qiagen, Valencia, CA). The beads were shaken on a Retsch 300 MM mixer mill with a frequency of