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Development of High-Throughput Method for Monitoring Food- and Water-Borne Pathogens

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Widespread concern about both bioterrorism and food poisoning by pathogens has led us to address the need for advanced diagnosis and surveillance techniques for food- and water-borne pathogens. Conventional detection methods, which involves successive cultures of the contaminating pathogens, is extremely labor-intensive and time-consuming (72 hr to presumptive identification), and unsuited for addressing microbial risk assessment during food processing and manufacture. Despite many developments over the last decades, no novel method has achieved the required sensitivity within a practical timescale (one working day). Rapid detection of pathogens would offer great commercial advantages to companies in the food industry as well as to general public health. Such a method would allow rapid identification of contaminated foods, allowing the decision to hold a batch of food products or allowing foods to be safely released.

The primary advantage to the application of molecular methods to the detection of pathogenic microorganisms from environmental and food samples are the ability to specifically and rapidly detect the target organisms of interest without having to actually isolate them on growth media. Given the large background of nonspecific microorganisms, the molecular methods hold promise for the future of monitoring for specific or selected groups of water- and food-borne pathogens. Our approach takes the advantages of the rapidly increasing amount of available pathogen sequence information, the power of nucleic acid amplification by PCR, and the broad-spectrum of DNA microarray that can query for more than thousands sequences simultaneously. Here, we outline the application strategies of pathogen-specific molecular targets and genus-/species-specific virulence genes and briefly describe the practical limitations of molecular detection methods.

A. Antibody-based methods

Fluorescently labeled antibodies specific for the target pathogen have been used to screen environmental samples (4). The biggest advantage of the antibody-based method is that a relatively inexpensive diagnostic kit enabling instant tests is possible and it can provide a mechanistic measure when using micro-titer plate reader (ELISA) with minor manipulation of the sample. However, there are practical difficulties for the antibody-based method to be applied to detect pathogens in the environmental samples. One should use monoclonal antibodies, since there are many background microorganisms in the sample and there may be some that share similar epitopes with which the antibody could cross-react. In addition, the surface antigens expressed by the target microorganism may differ depending on the conditions in its environment, since the target epitopes depend on the physiological state of the microorganism used to prepare the monoclonal antibody. The fatal disadvantage of the antibody-based method for our purposes is that the method lacks a required sensitivity. A minimum of 10^5 cells/ml are required to generate a clear response above background (2). The antibody-based method has not yet reached the sensitivity necessary for testing regulatory compliance, and nucleic acid-based method is considered to be superior to antibody-based methods for screening environmental samples (6).

B. Nucleic acid-based methods

1) Direct probing

If the target pathogens are in sufficient quantity in the sample, PCR amplification of the target nucleic acid specific for the target organism may not be necessary. In these instances, known nucleic acid probes specific for the target sequence can be used as a detection system after extracting total nucleic acids from the sample. This direct probing is more sensitive than the antibody-based method. The detection limit of conventional membrane hybridization (dot blotting) using either radioactive labeling and fluorescent labeling is generally femtogram quantities, and the direct probing method has been successfully used for detecting some pathogens (5). However, the direct probing is labor-intensive and time-consuming compared to the other nucleic acid-based methods, hence may not be appropriate for commercial diagnostic kit.

2) PCR

PCR is the most sensitive, inexpensive, and fast method for the purpose of pathogen detection. Even one copy of target sequence in a given template can be detected within 3~4 hr under ideal PCR condition. Moreover, PCR primers can be chosen from the public database without obtaining cultured strains. Once specific target sequences are determined, one can select the preferred primer sequences by searching literature or the sequences deposited in the database. Many software packages have been developed for scanning primer sequences from query sequences. Primer sets can be highly specific for only one particular gene that is unique to a single strain, or they can be universal, amplifying related sequences that cross

given taxonomic boundaries. In addition, data acquisition procedures can be much simplified by real-time monitoring of the PCR amplicon (real-time PCR), which eliminates post-PCR procedures. For portable and instant pathogen testing kit, PCR amplicon can be monitored by hybridization with immobilized probes. In spite of these advantages, the throughtput of PCR is very low, generally one target sequence in a reaction. Multiplex PCR enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers, and has been applied for multiple pathogen detection in environmental samples (1, 8). However, the maximum number of primer set is 3~4, and it is tedious and time-consuming to establish the multiplex assays, requiring lengthy optimization procedures.

3) DNA Microarray

DNA microarrays provide a powerful tool for the parallel analysis of many genes. Most DNA microarray studies conducted so far evaluate gene expression by competitive hybridizations between different populations of mRNA expressed under different culture conditions. The relative extents of hybridizations of target genes to probes on the microarray provide information on the degree of expression of genes of interest. DNA microarray technology holds promise for environmental microbiology (7), for example, detecting and quantifying different gene families involved in biogeochemical cycling, biodegradation, and pathogenesis in a high-throughput manner. However, the approaches used for gene expression analysis or other previously reported applications are inappropriate for the titration of genes or DNA sequences in environmental samples, because the probe sizes (printed spots) vary and evenness of hybridization cannot be assured. Hence, interpretation of hybridization profiles obtained from one-color hybridization (similar to conventional Southern hybridization) is not accurate. DNA microarray hybridization to quantify the amount of a gene requires direct binding of the target sequence to the probe DNA rather than competitive hybridizations, and the extent of this direct binding should be normalized against the concentration variations in the probe DNA and the spatial variation in the extent of hybridization (3). An alternative approach with a different microarray design and hybridization scheme is required to quantify target genes in environmental samples.

Considering the relative merits of the above-mentioned molecular approaches, we have concluded that the PCR amplification technique, when combined with DNA microarray technology, holds tremendous potential for developing improved tools for faster and more accurate detection of specific pathogens in the clinical and environmental samples. We propose the development of DNA microarray-based detection kits through comprehensive understanding of the biosafety-associated molecular makers, the DNA amplification and sample preparation procedures to optimize the DNA microarray hybridization technology. The topics discussed will include the updated information on the current biosafety marker technology and its future prospects.

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