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cDNA microarray screening in food safety

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Abstract

The cDNA microarray technology and related bioinformatics tools presents a wide range of novel application opportunities. The technology may be productively applied to address food safety. In this mini-review article, we present an update highlighting the late breaking discoveries that demonstrate the vitality of cDNA microarray technology as a tool to analyze food safety with reference to microbial pathogens and genetically modified foods. In order to bring the microarray technology to mainstream food safety, it is important to develop robust user-friendly tools that may be applied in a field setting. In addition, there needs to be a standardized process for regulatory agencies to interpret and act upon microarray-based data. The cDNA microarray approach is an emergent technology in diagnostics. Its values lie in being able to provide complimentary molecular insight when employed in addition to traditional tests for food safety, as part of a more comprehensive battery of tests.
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1. Introduction

Transcriptome profiling using the cDNA microarray approach and related bioinformatics tools presents a wide range of novel application opportunities. Recently, we (Liu-Stratton et al., 2004) and others (Kato et al., 2005) have reviewed the potential application of the cDNA microarray technology in the nutraceutical industry with emphasis on food safety. This article represents an update highlighting the late breaking discoveries that demonstrate the vitality of cDNA microarray technology as a tool to analyze food safety.

2. Assessment of microbial pathogens

DNA microarray technology offers an opportunity to examine the relationship between host and pathogen in much greater detail than has been possible previously. Optimal design of immobilized nucleic acids has a direct impact on the reliability of microarray results. It is now possible to appreciate the global changes in the transcriptome of a host cell or organism as a function of exposure to any given pathogen. In this way, it is possible to derive "signature" responses in the host that could diagnose the identity of an unknown pathogen. On the other hand, monitoring of microbial gene expression would enable the prediction of functions of uncharacterized genes, probe the physiologic adaptations made under various environmental conditions, identify virulence-associated genes, and test the effects of drugs. Thus, complete genomic sequences of microbial pathogens and hosts offer sophisticated new strategies for study-

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ing host–pathogen interactions. This new opportunity is being utilized to understand the mechanisms of pathogenesis and host specificity of specific pathogen (May et al., 2001). For example in the *Drosophila* genome sequencing project, microarray analysis and the use of genetic screens have led to the identification of several new genes required to combat microbial infection, filling in some important gaps in the understanding of innate immunity (Tzou et al., 2002).

Streptococcus pneumoniae remains a major causative agent of serious human diseases. The worldwide increase of antibiotic resistant strains revealed the importance of horizontal gene transfer in this pathogen, a scenario that results in the modulation of the species-specific gene pool. Genomic comparison between *S. pneumoniae* and commensal *Streptococcus mitis* and *Streptococcus oralis* strains identified distinct antigenic profiles and suggested a smooth transition between these species, supporting the validity of the microarray system as an epidemiological and diagnostic tool (Hakenbeck et al., 2001). *Helicobacter pylori* infect the stomachs of half of all humans. It has a relatively benign relationship with most hosts but produces severe pathology, including gastric cancer, in others. Microarray has been productively used as a tool to identify microbial genes that affect the magnitude of host responses to infection (Bjorkholm et al., 2002). An oligonucleotide microarray has been developed and used for the analysis of thermophilic *Campylobacter* spp., the primary food-borne pathogen in the United States (Volokhov et al., 2003).

Genomics research provides an unprecedented opportunity to probe into the pathogenicity and evolution of the world's most deadly pathogenic bacterium, *Yersinia pestis*. cDNA microarray has been successfully used as a tool to define the heat- and cold-shock stimulons in *Y. pestis*. These results provide a set of new candidate genes for hypothesis-based investigations of their roles in stress response, host adaptation and pathogenicity of this deadly pathogen (Han et al., 2005). Furthermore, microarray analyses in conjunction with PCR validation revealed that there are considerable genome dynamics, due to gene acquisition and loss, in natural populations of *Y. pestis* (Grimm et al., 2004). The opportunistic fungal pathogen *Candida albicans* is the major causative agent of oropharyngeal candidiasis in AIDS. The cDNA microarray technology enabled the characterization of differences in gene expression from a fluconazole-susceptible and a fluconazole-resistant well-characterized, clinically obtained matched set of *C. albicans* isolates to identify genes which are differentially expressed in association with azole resistance (Rogers and Barker, 2002). Microarray analysis proved to be effective in studying the molecular mechanisms of drug resistance in pathogenic organisms.

Standard clinical procedures for pathogen resistance identification are laborious and usually require two days of cultivation before the resistance can be determined unequivocally. In contrast, clinicians and patients face increasing threats from antibiotic-resistant pathogenic bacteria in terms of their frequencies and levels of resistance. Recently, it was observed that the oligonucleotide microarray technology outperformed the standard procedures in terms of assay time and the depth of information provided. The array system designed offered an attractive option for the identification and epidemiologic monitoring of TEM beta-lactamases in the routine clinical diagnostic laboratory (Grimm et al., 2004). Infection with *Mycobacterium tuberculosis* causes the illness tuberculosis with an annual mortality of approximately 2 million. Understanding the nature of the host–pathogen interactions at different stages of tuberculosis is central to new strategies for developing chemotherapies and vaccines. The microarray technology has been adapted to analyze the change in gene expression profiles of *M. tuberculosis* during infection in mice. Microarray analysis revealed clusters of genes that changed their transcription levels exclusively in the lungs over time (Talaat et al., 2004). This in vivo expression-profiling technology is applicable to any microbial model of infection. *Bacillus cereus*, *B. thuringiensis* and *B. anthracis* are closely related medically and economically important bacterial species that belong to the *B. cereus* group. Members of the *B. cereus* group carry genes encoding several important virulence factors, including enterotoxins, phospholipases and exotoxins. It is difficult to differentiate among *B. cereus* group members. A microarray-based tool to detect the virulence factor genes for strain identification and for determining virulence has been recently developed. The method requires an initial multiplex PCR amplification step, followed by identification of the PCR amplicons by hybridization to an oligonucleotide microarray containing genes for all three types of *Bacillus* virulence factors including *B. anthracis* virulence factors. The DNA chip contains 21 identical arrays and may be used for the analysis of seven samples in triplicates. This novel approach led to the discovery that virulence factors are present in several combinations in the strains analyzed (Sergeev et al., 2005).

The microarray technology is getting ready to come out of the laboratory and be a high throughput tool in the hands of end users. The potential is enormous and developments in that direction during the recent years are encouraging. One example is the procedure standardized for the monitoring of wastewater. Numerous waterborne

pathogens are difficult to detect and enumerate with accuracy due to methodological limitations and high costs of direct culturing. The purity of DNA extracted from wastewater samples is an important issue in the sensitivity and the usefulness of molecular methods such as polymerase chain reaction and hybridizations on DNA microarrays. Specific methods that would enable the microarray analysis of the genetic material of wastewater pathogens have been optimized (Lemarchand et al., 2005). Microarray-based assay systems are gradually becoming turn-key, lending themselves to applications outside the specialized molecular biology laboratories. Recently, an efficient approach for selecting 40–60 mer oligonucleotide probes combining optimal thermodynamic properties with high target specificity, suitable for genomic studies of microbial species has been described (Charbonnier et al., 2005). The algorithm for filtering probes from extensive oligonucleotides libraries fitting standard thermodynamic criteria includes positional information of predicted target-probe binding regions. This algorithm efficiently selected probes recognizing homologous gene targets across three different sequenced genomes of *Staphylococcus aureus*. BLAST analysis of the final selection of 5427 probes yielded >97%, 93%, and 81% of *S. aureus* genome coverage in strains N315, Mu50, and COL, respectively. A manufactured oligoarray including a subset of control *Escherichia coli* probes was validated for applications in the fields of comparative genomics and molecular epidemiology, mapping of deletion mutations and transcription profiling. This represents an example of the future of microarray applications – a standardized robust tool meant for the end user.

3. Microbial pathogens and food safety

Identification and prioritization of effective food safety interventions require an understanding of the relationship between food and pathogen from farm to consumption. Concerns about food safety have played a key role in the emergence of the public health system in the United States. Microbial risk assessment is a critical discipline in the area of food safety. The National Academy of Sciences risk assessment methodology for food-borne microorganisms consists of four steps: (1) disease characterization /hazard identification; (2) dose–response assessment; (3) exposure assessment; and (4) risk characterization. Potential hazards may be physical, artificial or naturally-occurring chemicals, organisms which cannot reproduce outside a specified life-cycle (e.g., parasites such as tapeworm in pigs) or viruses. Other microbes reproduce in the gastrointestinal tract of food animals as well as on the surface of food and in the environment. Methods of risk assessment for physical and chemical hazards have been used for many years. However, with microbial pathogens which can survive and grow on meat, in soil, water or other media, risk assessment methods are at the early stages of development. Because of the broad habitat range, the role of microbial pathogens in the food safety of meat, poultry, fruit and vegetables is important. The use of antibiotics in livestock species may accelerate the development of antibiotic-resistant strains of microbial pathogens, potentially complicating treatment for both animals and humans.

In 1995, basic changes were made in the structure of the U.S. food safety regulatory agencies. The USDA implemented the Pathogen Reduction: Hazard Analysis and Critical Control Point (HACCP) Systems. USDA's Food Safety and Inspection Service (FSIS) implemented the Final Rule for Meat and Poultry. In addition, FoodNet, a sentinel surveillance system for active collection of food-borne disease surveillance data, was developed (Morris, 2003). The U.S. Department of Agriculture has established standards for the composition and shelf stability of various ready-to-eat meat products. These standards may include product pH, moisture:protein ratio, and water activity (aw) values. HACCP, risk assessment, predictive microbiology, and dose-response modeling have been recognized as important tools for the assessment and management of health risks posed by food-borne pathogens (McNab, 1998). The important new concept of the food safety objective (FSO) offers a strategy to translate public health risk into a definable goal such as a specified maximum frequency or concentration of a hazardous agent in a food at the time of consumption that is deemed to provide an appropriate level of health protection (Szabo et al., 2003). The overall food-pathogen testing market is expected to grow to $192 million and 34 million tests by 2005 (Alocilja and Radke, 2003).

4. The microarray technology and food safety

The potential of oligonucleotide microarrays for medical, food safety and biodefense analysis of microbial pathogens is fast unfolding. Microarray analysis is an emerging technology that has the potential to become a leading trend in bacterial identification in food and feed improvement (Al-Khaldi et al., 2002). The DNA microarray technology has been employed to compare the gene expression profiles in liver among three groups of mice fed a diet containing 5% royal jelly, a diet containing 5% royal jelly stored at 40 °C for 7 d (40

– 7 d royal jelly) or a control diet which provides the same total energy as royal jelly. The results suggest that the efficacy of royal jelly decreased and the toxicity of royal jelly increased during storage at high temperature (Kamakura et al., 2005). These findings suggest that the application of DNA microarray technology to the biochemical evaluation of food safety may be effective for rapid and precise quality control. *Salmonella* spp. represent one of the most problematic food pathogens in public health, as they are responsible for food poisoning associated with contamination of meat, poultry, and eggs. The genotype of *Salmonella enterica serovar Enteritidis* was correlated with the phenotype using DNA-DNA microarray hybridization, ribotyping, and phenotype microarray analysis to compare three strains that differed in colony morphology and phage type. These studies led to the observation that the pandemic of egg-associated human salmonellosis that came into prominence in the 1980s is primarily an example of bacterial adaptive radiation that affects the safety of the food supply (Morales et al., 2005). Rapid and sensitive detection of *Salmonella* spp. is required to ensure food safety. Recently, a fiber-optic DNA microarray using microsphere-immobilized oligonucleotide probes specific for the *Salmonella invA* and *spvB* genes was developed for the detection of *Salmonella* spp. (Ahn and Walt, 2005). Microarrays were prepared by randomly distributing DNA probe-functionalized microspheres (3.1-micron diameter) into microwells created by etching optical fiber bundles. Hybridization of the probe-functionalized microspheres to target DNA from *Salmonella* was performed and visualized using Cy3-labeled secondary probes in a sandwich-type assay format. The DNA microarray was specific and showed no cross-reactivity with other common food pathogens, including *E. coli* and *Y. enterocolitica*, and could even detect *Salmonella* spp. from cocktails of bacterial strains with only moderate loss of sensitivity due to nonspecific binding. Fiber-optic DNA microarrays can be used for rapid and sensitive detection of *Salmonella* spp. Since fiber-optic microarrays can be prepared with different probes, this approach could also enable the simultaneous detection of multiple food pathogens (Ahn and Walt, 2005).

A disposable microarray (ArrayTubes) has been developed for the detection of up to 90 antibiotic resistance genes in gram-positive bacteria by hybridization. Each antibiotic resistance gene is represented by two specific oligonucleotides chosen from consensus sequences of gene families, except for nine genes for which only one specific oligonucleotide could be developed. A total of 137 oligonucleotides (26–33 nucleotides in length with similar physicochemical parameters) were spotted onto the microarray. The ArrayTube platform presents the advantage of rapidly screening bacteria for the presence of antibiotic resistance genes known in Gram-positive bacteria (Perreten et al., 2005). The more generic GeneChip DNA microarray system has been utilized to test food safety by comparing the gene expression profiles among rats fed on 12% casein, 12% gluten, and protein-free diets for one week (Kato et al., 2004). A gene-specific DNA microarray technique has been developed for the comprehensive detection of pathogenic vibrios that are natural inhabitants of warm coastal waters and shellfish. Multiplex PCR with vvh and viuB for Vibrio vulnificus, with ompU, toxR, tcpI, and hlyA for *V. cholerae*, and with tlh, tdh, trh, and open reading frame 8 for *V. parahaemolyticus* helped to ensure that total and pathogenic strains, including subtypes of the three *Vibrio* spp., could be detected and discriminated. For DNA microarrays, oligonucleotide probes for these targeted genes were deposited onto epoxysilane-derivatized, 12-well, Teflon-masked slides by using a MicroGrid II arrayer. This microarray-based assay has the potential to ensure rapid and accurate detection of pathogenic vibrios in shellfish, thereby improving the microbiological safety of shellfish for consumers (Panicker et al., 2004). *Campylobacter* is the most common cause of human acute bacterial gastroenteritis worldwide, widely distributed and isolated from human clinical samples as well as from many other different sources. To comply with the demands of consumers for food safety, there is a need for development of a rapid, sensitive and specific detection method for *Campylobacter*. A novel DNA-microarray based detection method has been recently reported (Keramas et al., 2003). The technique has been utilized to evaluate *Campylobacter* and non-*Campylobacter* reference strains and to detect *Campylobacter* directly from the faecal cloacal swabs. This technique lends itself to automation and incorporation into a dedicated mass screening microsystem (Keramas et al., 2003).

The safety of foods derived from genetically modified organisms has been a great concern since the first introduction of genetically modified tobacco in 1998. The International Food Biotechnology Council (IFBC) published the first report on this issue over a decade ago (IFBC, 1990). Since then, several organizations, including the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the Organisation for Economic Cooperation and Development (OECD) and the International Life Sciences Institute (ILSI), have provided guidelines for the safety assessment of genetically modified foods. The concept

of substantial equivalence has been used as the comparative approach to assess the safety of genetically modified foods. The substantial equivalence is based on the idea that traditional foods, which are considered safe due to a long history of use, can serve as a reference to compare their genetically modified counterpart. This concept helps to identify similarities and differences between the traditional foods and the genetically modified products.

Several safety issues of genetically modified foods should be considered. The process of genetic modification, the safety of new proteins synthesized, the potential occurrence of unintended effects, the possible gene transfer to human intestinal flora, the potential allergenic and/or toxic effects of new protein, and the role of new foods in the diet and the influence on food processing (Kuiper et al., 2001). The same gene located in different types of cells/tissues may produce the same protein manifesting a contrasting function. Therefore, introduction of a gene to two different types of organisms may produce different functional outcomes presenting the risk of adverse effects. Due to gene–gene interactions, the insertion of a new gene may also change the gene expression profile, enzymatic and metabolic pathways, and consequently impact the phenotype of the recipient cells. For instance, the over-expression of a polygalacturonase gene that was involved in the pectin synthesis had no apparent effect on tobacco (Osteryoung et al., 1990). However, the same gene changed the phenotype, including silvery colored leaves and premature leaf shedding, in apple trees (Atkinson et al., 2002). Introduction of a new gene may trigger production of new molecules that are potentially toxic, allergenic, or carcinogenic unless proven otherwise. For example, L-tryptophan produced by transgenic Showa Denko KK bacteria caused the 1989 epidemic of eosinophilia-myalgia syndrome (EMS) in the United States due to the trace contaminants produced by the transgenic bacteria (Kilbourne et al., 1996). The unintended effects of certain genetically modified foods have been well documented (Murrey et al., 1999; Shewmaker et al., 1999; Ye et al., 2000). For example, rice that was genetically modified to promote provitamin A biosynthetic pathway produced unexpected carotenoid derivatives such as beta-carotene, lutein, and zeaxanthin (Ye et al., 2000). Wheat genetically modified to increase the expression of glucose oxidase has exhibited phytotoxicity (Murrey et al., 1999).

The concerns addressed above warrant targeted studies to compare genetically modified food and their traditional counterparts. Because the introduced gene(s) are likely to impact gene expression patterns and downstream metabolic pathways, it would be prudent to study the gene expression profiles of genetically modified foods as well as of tissues from experimental humans and animals who have ingested such food with the corresponding traditional food as reference. A global approach to gene expression analysis will provide critical additional information especially because it is almost impossible to predict the genetic response in such a complex experiment. Under these conditions, not only changes in gene expression but no-change responses would be equally meaningful because one would then be assured of the specific sets of genes that are not sensitive to the specific genetic modification of the food in question.

Taken together, the DNA microarray technology is highly applicable to the study of food safety. In order to bring the microarray technology to mainstream food safety it is important to develop robust user-friendly tools that may be applied in a field setting. In addition, there needs to be a standardized process for regulatory agencies to interpret and act upon microarray-based data. At present, it seems that regulatory agencies believe microarray data could be extremely useful in testing prospective chemical products and investigating safety problems after chemicals have been marketed. However, it has not yet been clearly established how this information will be used by agencies in the approval of pharmaceuticals and other chemicals–or even whether companies will be required to submit microarray data. Although some private companies are already voluntarily submitting microarray data along with their drug and pesticide applications, others are hesitant to do so. Most of the stakeholders involved agree that standardization of microarray experiment procedures and of genomic signatures are key to the broad acceptance and use of these data (Freeman, 2004). The cDNA microarray approach is an emergent technology in diagnostics. Its values lie in being able to provide complimentary molecular insight when employed in addition to traditional tests for food safety as part of a more comprehensive battery of tests.

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