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Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Review

DNA microarrays and their applications in medical microbiology

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Rapid diagnosis and treatment of disease is often based on the identification and characterization of causative agents derived from phenotypic characteristics. This can be laborious and time consuming, often requiring many skilled personnel and a large amount of lab space. However, the introduction of nucleic acid amplification techniques into molecular biology has transformed the laboratory detection of pathogens. The progression of the molecular diagnostic revolution currently relies on the ability to efficiently and accurately offer multiplex detection and characterization for a variety of infectious disease pathogens. DNA microarray analysis has the capability to offer robust multiplex detection. Multiple microarray platforms exist, including printed double-stranded DNA and oligonucleotide arrays, in situ-synthesized arrays, high-density bead arrays, electronic microarrays, and suspension bead arrays. The aim of this paper was to review DNA microarray technology, highlighting two major types: the oligonucleotide-based array and the PCR product-based array. Although, the use of microarrays to generate gene expression data has become routine, applications pertinent to microbiology continue to rapidly expand. This review highlights uses of microarray technology that impact diagnostic microbiology, including the detection and identification of pathogens, determination of antimicrobial resistance, epidemiological strain typing, and determination of virulence factors.

Key words: DNA microarray, applications, microbiology.

INTRODUCTION

The large-scale genome sequencing effort and the ability to immobilize thousands of DNA fragments on a surface, such as coated glass slide or membrane, have led to the development of DNA microarray technology (Cassone et al., 2006). An entire microbial genome can be easily represented in a single array, making it feasible to perform genome-wide analysis (Ye et al., 2006; Akondi and Lakshmi, 2013). The two common applications of DNA microarray technology in molecular biology are the exploration of genome-wide transcriptional profiles and the measurement of the similarities or differences in genetic contents among different microbes (Peterson et al., 2010). DNA microarray technology is being used to study many bacterial species ranging from standard laboratory strains and pathogens to environmental isolates (Murakami et al., 2002).

DNA microarrays are basically a miniaturized form of dot blot, but in a high-throughput format. There are two major types of DNA microarrays; one is the oligonucleotide-based array and the other is the PCR product-based array (Panicker et al., 2004). A DNA microarray experiment consists of array fabrication, probe preparation, hybridization and data analysis (Call et al., 2001). Although the basic array technology is the same, there are fundamental differences in its application to prokaryotes and eukaryotes. For example, total RNA is usually labeled for a bacterial array experiment, while poly(A) RNA is often used for eukaryotic arrays.

Detection of single bacterial genes (for example antibiotic resistance genes or species-specific genes) in

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diagnostics and in epidemiological studies is typically carried out by PCR, whereas DNA microarrays have been developed to perform a large number of different hybridization experiments simultaneously on a single membrane or glass substrate. They are well-suited to comprehensively investigate and quantitatively compare the expression levels of a large number of genes, but they can also be easily used in qualitative studies to detect selected DNA sequences (Call et al., 2003a; Call et al., 2003b; Perreten et al., 2005).

Simply defined, a microarray is a collection of microscopic features (most commonly DNA) which can be probed with target molecules to produce either quantitative (gene expression) or qualitative (diagnostic) data (Miller and Tang, 2009; Yu et al., 2013). Although other types of microarrays exist, such as protein microarrays (Lopez and Pluskal, 2003; MacBeath, 2002), this review will focus on DNA microarrays.

With the availability of complete genome sequences of many microorganisms, the DNA microarray technology has become a very powerful tool to explore global gene expression profiles and to measure genome-wide differences in genetic contents. Since only the abundance of transcripts or presence and absence of DNA regions are measured with a DNA microarray, interpretation of the array data can be difficult in the absence of other supporting evidence. This is especially true when the physiological events are not well studied. In addition, it is not easy to sort out secondary effects caused by mutations, expression of certain genes, and different growth conditions.

As a result, the greatest impact of this technology will not be realized until it is combined with other high-throughput genomic methods, biochemistry, genetics and physiology. Analysis of a systematically perturbed metabolic network in yeast clearly demon-strates the power of an integrated approach to build, test, and refine a model of a cellular metabolic pathway.

PCR PRODUCT-BASED DNA MICROARRAYS

Primer design

The first step of DNA microarray construction for microbes with known genome sequences is the design of primers to amplify specific regions of interest. In a bacterial genome, there are open reading frames (ORFs) and intergenic regions. ORFs are potential protein coding regions identified by computer analysis or experimentally. The intergenic regions include promoters or regions encoding small RNA molecules which may have regulatory functions (Lease and Belfort, 2000).

An ideal array should contain both ORFs and intergenic regions, although most of the current PCR product-based arrays only contain ORFs. Primer design for the whole genome can be carried out with a computer program such as PrimeArray (Raddatz et al., 2001). PrimeArray is specifically designed to compute the oligonucleotide primer pairs for genome-scale gene amplification. The simplest way to design primers is to use the beginning and ending regions of a specific ORF. If an ORF is too long (>3.0 kb), primers can be designed to reduce the size of the PCR product. Since there are repeated regions in a genome, amplification of unique sequences is necessary to avoid cross-hybridization. Before proceeding to the making of all the primers for the whole genome, it is important to test a portion of the primer sets to ensure the primer quality and desirable amplification results.

PCR amplification

The goal of the whole genome PCR amplification is to achieve the highest success rate and yield in a high-throughput manner. Conditions for PCR amplification are initially optimized with a few 96-well plates. The optimized conditions vary with genomes since they could have different GC contents and secondary structures. After completion of the first round of PCR for all the plates, further optimization or redesign of primers for failed reactions is necessary.

To reduce chromosomal DNA contamination and increase yield, some arrays are constructed with second round PCR products (Wei et al*.,* 2001). This practice may not be necessary as long as the amount of genomic DNA template remains low. In fact, the second round of amplification could increase the number of reactions that have multiple bands.

PCR product purification

To remove unincorporated nucleotides and primers, it is recommended to purify the PCR product. PCR purification can be done in either 96- or 384-plate format by ethanol precipitation or by commercial purification systems (Millipore, Qiagen, Whatman). The 96-well multiscreen filter plates from Millipore have been found to give excellent DNA product recovery with no significant contamination at a relatively low cost (Hegde et al., 2000).

If the goal is to perform high-throughput analysis for gene discovery, purification of PCR products is not necessary. The binding efficiency of PCR products and the quality of the array may be slightly compromised, but there is a significant saving in time and money

Spotting

The purified PCR products are spotted onto membranes or coated glass slides. DNA microarrays on coated glass

slides are prepared by printing DNA products with high-speed robots. The arraying robots can be custom made or purchased from commercial sources. The common problems associated with glass slides are spot morphology, high background, and batch variability. Although there are no perfect slides, aminosilane-coated slides (Corning, Telechem, Amersham Pharmacia Biotech) and poly-L- lysine-coated slides are commonly used. The PCR products are resuspended in an appropriate solution before spotting.

The two common solutions are high-salt buffer (3×SSC) and 50% dimethyl sulfoxide (DMSO). One of the factors in determining which chemistry to use is the type of slides. For CMT-GAPS aminosilane-coated glass microscope slides, DMSO has been found to offer many advantages (Hegde et al., 2000). It is hygroscopic and has a low vapor pressure, which allows DNA to be stored for long periods of time without significant evaporation.

After spotting, it is often necessary to check the quality of the slides and spotting results. This can be routinely performed with SYBR green staining (Battaglia et al., 2000) by hybridizing the array with a Cy-labeled genomic DNA or random 9-mer sequence. The SYBR green dye can be used to measure the amount of DNA in each spot and the spotting integrity, while the genomic DNA hybridization is a better indicator of the hybridization background and the quality of the slides (Ye et al., 2001).

Total RNA labeling

The cDNA probes for array hybridization are synthesized from total RNA by reverse transcriptase. The nucleotides can be labeled with radioisotopes (such as P^{32} orthophosphate) or fluorescent markers. Either random primers or specific primers are used in the reaction. The amount of total bacterial RNA used varies with the organism, stage of growth, type of array and labeling method. Typically, 7 to 15 µg of total RNA in combination with 6 µg of random hexamers generally yields good labeling efficiency and reasonable signal intensity with Cy5 or Cy3 fluor for arrays on glass slides. The incorporation efficiencies of Cy5- and Cy3-labeled nucleotides are not equal.

A two-step labeling procedure using aminoallyl-dUTP is gaining popularity due to the increased labeling efficiency and reduction in dye bias and cost. In this two-step procedure, primary aliphatic amino groups are first incorporated during cDNA synthesis. In the second step, the monofunctional *N*-hydroxylsuccinimide-activated fluorescent dye (Cy3, Cy5) is coupled to cDNA by chemical reaction with the amino functional groups.

Since the substrate for the reverse transcriptase is identical for all the samples and is less bulky, the two-step method could yield equivalent molarities of labeled probe with higher efficiency than the one-step labeling procedure (Call et al., 2001b). After labeling, it is necessary to remove the unincorporated dyes to reduce background. This can be achieved by conventional DNA purification methods. To insure the probe quality, the labeling efficiency for Cy3 and Cy5 needs to be calculated. The calculation is based on the extinction coefficient with the following formula: ($O.D._{550}$×dilution factor×total volume)/0.15 for Cy3, or ($O.D._{650}$×dilution factor×total volume)/0.25 for Cy5. The total amount of incorporated dye obtained is in pmol (Ye et al., 2001).

It is worthwhile to point out that there are other fluorescent markers, for example, Alexa fluor from Molecular Probes (Nsofor et al., 2013) or labeling methods that are available or being developed.

Genomic DNA labeling

Genomic DNA probes can be used for normalization, slide quality control, antibiotics resistance gene profile and comparative genomic studies. Genomic DNA can be labeled by nick translation or by random priming with the Klenow fragment of DNA polymerase. Direct chemical labeling of nucleic acids is also a commonly used method.

For example, the Universal Linkage System (ULS) is a technique for binding any marker group or label to DNA and RNA (Kreatech Diagnostics, Amsterdam, The Netherland). For the random priming method, the genomic DNA samples are first sheared by mechanical means such as nebulization or sonication. DNA fragments within 1-3 kb are collected and labeled by either the one- or two-step labeling procedure. A labeling reaction with 0.5-2 µg of genomic DNA often yields enough probes for a single hybridization experiment.

Hybridization and data acquisition

The amount of probe used for hybridization depends on the array format and labeling method. For arrays on glass slides, a reasonable signal to background ratio can be obtained with probes containing 100-200 pmol of incorporated fluorescent dye. In a typical hybridization reaction, equal amounts of Cy3- and Cy5-labeled probes based on the incorporated dye concentration are combined (Ye et al., 2000). To correct for the difference in labeling efficiency of Cy3 and Cy5, a dye swap procedure is used. In other words, the two samples are labeled with opposite dyes and the resulting probes are hybridized to two different slides.

The overall procedure for a PCR product-based DNA microarray hybridization is basically the same as for a Southern blot except for a few modifications. Before hybridization, most glass slides need to be treated to block or inactivate the non-specific binding sites. The procedure employed depends on the slide type and spotting chemistry. For aminosilane-coated slides, a pre-

prehybridization solution containing 1% BSA, 5×SSC and 0.1% SDS has been found to be effective (Hegde et al., 2000). Usually, the hybridization solution containing the probe is placed onto the array and covered with a cover slip. The glass slide is then placed in a humidified chamber. The temperature of hybridization and washing conditions depend on the GC content of the organism. Bacteria with high GC content require a more stringent washing condition in order to minimize non-specific binding.

After hybridization, the signal intensities of all the spots on a glass slide are captured by scanners (GSI Lumonics, Molecular Dynamics, Genomic Solutions, Axon, and others). For membrane arrays hybridized with P^{32}-labeled probes, a phosphor imaging system (Molecular Dynamics) can be used. Processing of array images involves three steps: spot finding, quantification, and background estimation. These steps are performed with the software provided by the scanner vendors or by other sources. In Nsofor et al., 2013), Applied Precision arrayWoRx scanner for image capturing and Applied Precision SoftWoRx Tracker software for processing and analysis of array images was used.

Data normalization

There are several systematic variables in a DNA microarray experiment that can affect the measurement of mRNA levels making direct comparisons. Sources of the variations include inherent errors from sample handling, slide to slide variation, difference in labeling or hybridization efficiency, and variations during image analysis. These differences are not due to the actual changes in gene expression levels. Normalization is a process of minimizing these variations, establishing a common base for comparison. Normalization can be done within the slide to adjust the dye incorporation efficiency, between the two slides for dye swap experiments and across slides for replicates of the same experiment (Yang et al., 2001). After normalization, the ratio is calculated for each spot on the slide.

OLIGONUCLEOTIDE-BASED DNA MICROARRAYS

Instead of using PCR products, DNA microarrays can be constructed with short oligonucleotides. In the Affymetrix system, Oligonucleotide is synthesized *in situ* on a derivatized glass surface using a combination of photolithography and combinatorial chemistry. The *Escherichia coli* Genome Array system by Affymetrix uses a protocol for the enrichment and labeling of the non-polyadenylated mRNA of prokaryotes. The mRNA is directly labeled so that it represents the natural distribution of RNA species within the sample. No reverse transcription or amplification steps are involved. On the other hand, the enrichment procedure could also potentially alter the mRNA population.

Selinger et al. (2000) have reported the application of a 30-base pair resolution *E. coli* genome array for RNA expression analysis using the Affymetrix system. This array contains on average one 25-mer oligonucleotide probe per 30 base pairs over the entire genome, with one every six bases for the intergenic regions and every 60 bases for 4290 ORFs. Twofold concentration differences can be detected at levels as low as 0.2 mRNA copies per cell. The array also permits the investigation of intergenic regions of the genome.

A system using one optimized 70-mer probe per gene has been developed by Operon Technologies (http://www.operon.com). It is similar to a PCR-based microarray except that no amplification is required. Another type of array has also been fabricated by immobilizing oligonucleotides in a polyacrylamide gel (Proudnikov et al., 1998).

Overall, the oligonucleotide-based DNA microarray has many advantages: (i) no amplification is required, and thus, there are no failed amplifications. It is difficult to obtain a high success rate of amplification for micro-organisms that contain high GC content or complex DNA structure; (ii) there are fewer chances for contamination due to non-specific amplification and mishandling; (iii) There is a reduction in cross-hybridization and an increase in the differentiation of overlapping genes or highly homologous regions. (iv) it is easier to normalize concentrations of oligonucleotides; (v) high-density oligonucleotide arrays enable high coverage of the genome, and thus, allow a precise mapping of the transcriptional regions and identification of alternative promoters. However, the cost of making long oligonucleotides is high. There are a limited number of whole microbial genome arrays that are available in the Affymetrix system (Proudnikov et al., 1998).

DNA microarray database

DNA microarray experiments generate vast amounts of data. The goal of the array database is to allow researchers to retrieve, analyze and visualize the array data. It can also serve as a means to link array data to other information, such as DNA and protein sequences, protein expression profiles and cellular function. In addition, an array database will make it possible to compare gene expression profiles across microbial species.

The EcoReg website (http://www.genomics.lbl.gov/~ecoreg/) was designed to be a repository of primary data (analogous to Genbank) for bacteria transcriptional control processes. It is a bioinformatics database project to facilitate improved understanding and modeling of the transcriptional control of bacteria gene expression.

There are commercial software packages available to facilitate database construction. For example,

GeneSpring Suite™ from Silicon Genetics includes a web database and other tools for data sharing. The SCOUT platform developed by Lion Bioscience is based on a sequence retrieval system (SRS). It integrates the array data analysis package, arraySCOUT, to other analysis tools and databases. They include bioSCOUT for automated gene and genome analysis, pathSCOUT for metabolic pathway analysis, and πSCOUT for analysis of protein-protein interaction. This integration also allows a sharing of data on an enterprise-wide level (Call et al., 2001).

Validation of DNA microarray data

There are three major sources of errors associated with the application of DNA microarrays: initial construction of the print-ready plates, array experiment, and data analysis. During the construction of the print-ready plates, there are handling issues such as plate transfer, which could lead to cross-contamination or other types of mishandling. During PCR amplification, the presence of multiple bands can lead to false results (Call et al., 2003).

Mistakes can also come from the generation of the final gene list, leading to a mismatch between the clone and spot position on the final array. As a result, it is advisable to check the hybridization results against well-characterized genes and internal controls when a new set of spotting plates is made.

The common experimental errors can stem from uneven hybridization, inefficient labeling and problems during RNA preparations. During data analysis, it is difficult to have an accurate ratio calculation when the signal intensity is low. When the fold of induction is low, the result can be misinterpreted. All these issues lead to the need for validation of the array experiment. Supporting evidence can be obtained from enzymatic assays, reporter gene systems, and other direct RNA quantification methods such as quantitative PCR (qPCR), nuclease protection assay, and primer extension. qPCR (Applied Biosystems, http://www.appliedbiosystems.com/) offers a high-throughput advantage and can be an excellent tool to supplement array analysis. As with any qPCR reaction, proper controls need to be implemented and care must be taken to avoid DNA contamination in RNA samples.

When the results of a DNA microarray experiment were compared with those obtained from a Northern blot, the sensitivity of the DNA microarray was found to be slightly less than that of a Northern blot analysis (Taniguchi et al., 2001). In most genes, the data obtained by the two methods were consistent.

However, in 4 of 46 genes compared, the DNA microarray failed to detect the expression changes that were revealed by the Northern blot. The data demonstrated that DNA microarrays provide quantitative data that are comparable to the Northern blot in general.

APPLICATIONS OF DNA MICROARRAYS IN MEDICAL MICROBIOLOGY

Microbial detection and identification

Perhaps the most promising area in applying DNA microarray technology in medical microbiology is its use for simultaneous assessment of large numbers of microbial genetic targets (Stover et al., 2003; Gentry and Zhou, 2006). Specific microbial gene amplification by either a broad-range or a multiplex PCR prior to micro-array analysis enhances test sensitivity. The amplification of universal microorganism targets by broad-range PCR followed by sequencing analysis has been considered a standard procedure (Tang et al., 1998); however, microarrays have emerged as potential tools for bacterial detection and identification given their high parallelism in screening for the presence of a wide diversity of genes. The most commonly used gene targets have been the 16S bacterial and 28S fungal and intergenic transcribed spacers (ITSs) in rRNA genes, and microarray technology has been incorporated to compensate for the time-consuming sequencing identification procedure (Tang et al., 1998). An oligonucleotide microarray targeting the 16S rRNA gene was developed for the detection of a panel of 40 predominant human intestinal bacterial pathogens in human fecal samples (Wang et al., 2004; Wapner et al*.,* 2012). Assays using broad-range PCR incorporated with microarrays have been shown to allow rapid bacterial detection and identification with positive blood cultures (Anthony et al., 2000; Marlowe et al., 2003). A similar procedure was developed and used for the rapid diagnosis of bloodstream infections caused by common bacterial pathogens in the pediatric and general populations (Shang et al., 2005; Cleven et al., 2006). PCR amplification, in combination with an oligonucleotide microarray, was used to identify *Bacillus anthracis* based on the rRNA ITS region (Nubel et al., 2004; Roh et al., 2012). Several studies reported the use of microarrays to identify pathogenic yeasts and molds by targeting the ITS regions in fungal rRNA genes (Hsiao et al., 2005; Huang et al., 2006). In another study, a DNA microarray was established to detect and identify 14 commonly encountered fungal pathogens in clinical specimens collected from neutropenic patients (Spiess et al., 2007; McLoughlin, 2011).

The key for broad-range PCR amplification followed by microarray identification to work is to target the right gene. It is critical to use a gene "broad" enough so that most related microorganisms can be covered in one amplification reaction. On the other hand, the targeted gene should possess enough polymorphic information to supply sufficient discriminatory power to differentiate and characterize related microorganisms. Degenerate primer sets can be designed to increase the coverage of relatively variable genes. Other universal bacterial genes have been used to detect and identify organisms using

microarrays.

For mycobacterial detection and identification, the *gyrB*, *rpoB*, and *katG* genes have been targeted by using microarrays (Fukushima et al., 2003). Microarrays targeting the 23S rRNA and *gyrB* genes for bacterial detection and identification using clinical specimens have been described (Kakinuma et al., 2003; Kostic et al., 2007). In addition to bacterial and myco-bacterial organisms, microarrays following broad-range PCR amplification have been used to detect and identify fungal, parasitic, and viral pathogens (Diaz and Fell, 2005; Korimbocus et al., 2005; Wang, et al., 2005).

Microarrays have also been incorporated with multiplex PCR amplification for the simultaneous detection and identification of a panel of microbial pathogens in a single reaction. Khodakov et al. (2008) described a novel microarray-based approach for the simultaneous identification and quantification of human immunodeficiency virus type 1 (HIV-1) and hepatitis B and C viruses in donor plasma specimens (Khodakov et al., 2008). A microarray technique for the detection and identification of enteropathogenic bacteria at the species and subspecies levels was developed, covering pathogenic *E. coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella enterica*, *Campylobacter jejuni*, *Shigella* spp., *Yersinia enterocolitica*, and *Listeria monocytogenes* (You et al., 2008).

A microarray-based multiplexed assay was developed to detect foot-and-mouth disease virus with rule-out assays for two other foreign animal diseases and four domestic animal diseases that cause vesicular or ulcerative lesions that are indistinguishable from those of foot-and-mouth disease virus infection of cattle, sheep, and swine (Lenhoff et al., 2008). Bøving et al. (2009) developed a novel multiplex PCR with product detection by the Luminex suspension array system covering a panel of bacterial and viral pathogens causing meningitis. This system detected and identified nine microorganisms including *Neisseria meningitidis*, *Streptococcus pneumoniae*, *E. coli*, *Staphylococcus aureus*, *L. monocytogenes*, *Streptococcus agalactiae*, herpes simplex virus types 1 and 2, and varicella zoster virus directly from cerebrospinal fluid (Bøving *et al.,* 2009). The ResPlex I system, manufactured by Qiagen (Valencia, CA), was used to detect a panel of bacterial pathogens related to community-acquired pneumonia from tracheal aspirates collected from hospitalized antibiotic-treated children. The data indicated that the ResPlex I system significantly enhanced the pathogen-specific diagnosis of community-acquired pneumonia in children (Deng et al., 2009).

Comparative genomics and microbial typing

Genomic hybridization of a whole genome array detects the presence or absence of similar DNA regions in other microorganisms, allowing genome-wide comparison of their genetic contents. It is an effective way to conduct a comparative genomic study in the absence of complete genome sequences. DNA microarrays have been used to investigate genome differences between *M. tuberculosis*, *M. bovis* and the various Bacille Calmette-Guérin (BCG) daughter strains (Behr et al., 1999) within the species of *H. pylori* and *M. tuberculosis* (Kato-Maeda et al., 2001) among different isolates of *S. pneumoniae* (Hakenbeck et al., 2001). These studies show that DNA microarrays can facilitate a better understanding of the genetic differences between closely related organisms, providing useful information for the identification of virulence factors, exploration of molecular phylogeny, improvement of diagnostics and development of vaccines.

DNA microarray technology is also an excellent way to identify changes in genetic content of the same strain after long-term adaptation or strain optimization. After adaptation for 2000 generations to a stressful high temperature of 41.5°C, *E. coli* was examined on a genome-wide scale for duplication/deletion events by using DNA arrays (Riehle et al., 2001). A total of five duplication and deletion events were detected, providing additional evidence for the idea that gene duplication plays an integral role in adaptation, specifically as a means for gene amplification.

Numerous studies that use DNA microarrays for microorganism typing by taking advantage of its simultaneous detection of a variety of genomes have been reported. The accurate identification and prompt typing of pathogens causing diarrheal diseases are critical for directing clinical intervention, including appropriate antibiotic administration, and facilitating epidemiological investigations. Microarray-based approaches along with other genetic approaches that can be used to support or replace the classical serotyping method for several conventional diarrhea bacterial pathogens have already been offered. The use of microarrays has included *Salmonella*, *Helicobacter*, and *Campyloba cter* species (Fitzgerald et al., 2007; Salama et al., 2000; Volokhov et al., 2003 Willse et al., 2004). PCR followed by a microarray hybridization step has been used for the detection and typing of *E. coli* virulence genes (van Ijperen et al., 2002). A serotype-specific DNA microarray for the identification of clinically encountered *Shigella* and pathogenic *E. coli* strains has being described (Li et al., 2006). Diagnostic microarrays based on the ArrayTube format were devised for virulence determinant detection as well as for protein-based serotyping of *E. coli* (Korczak et al., 2005; Anjum et al., 2007). A novel ArrayTube assay, which incorporates oligonucleotide DNA probes representing 24 of the most epidemiologically relevant O antigens and 47 H antigens, has been described for fast DNA serotyping of *E. coli* (Ballmer et al., 2007). Microarrays have also been used to characterize and type other gastroenteritis-causing viral pathogens including rotavirus, norovirus, and

astrovirus (Chizhikov et al., 2002; Honma et al., 2007; Jaaskelainen and Maunula, 2006; Lovmar et al., 2003). Beyond diarrheal illnesses, Pas et al. (2008) reported the comparison of reverse hybridization, microarray, and sequence analysis for hepatitis B virus (HBV) genotyping, suggesting that the InnoLipa HBV genotyping strip assay, a microarray-based system, detected dual infections and was an easy and quick tool for HBV genotyping.

Determination of virulence factors

Many genes associated with virulence are regulated by specific conditions. One way to determine the candidate virulence factors is to investigate the genome-wide gene expression profiles under relevant conditions, such as physiological changes during interaction with the host. A second approach would rely on comparative genomics. In a genome comparison study among *H. pylori* strains, a class of candidate virulence genes was identified by their coinheritance with a pathogenicity island (Salama et al., 2001). The whole genome microarray of *H. pylori* was also shown to be an effective method to identify differences in gene content between two *H. pylori* strains that induce distinct pathological outcomes (Israel et al., 2001). It was demonstrated that the ability of *H. pylori* to regulate epithelial cell responses related to inflammation depends on the presence of an intact *cag* pathogenicity island.

Gene expression profiles of drugs, resistance, inhibitors and toxic compounds

Inhibition of a particular cellular process may result in a regulatory feedback mechanism, leading to changes in gene expression patterns. Exploring the gene expression profiles with DNA microarrays may reveal information on the mode of action for drugs, resistance, inhibitors or toxic compounds. DNA microarray hybridization experiments have been conducted in *M. tuberculosis* to explore the changes in gene expression induced by the antituberculous drug isoniazid (INH) (Wilson et al., 1999). INH selectively interrupts the synthesis of mycolic acids, which are branched β-hydroxy fatty acids. Microarray experiments showed that isoniazid induced several genes that encode proteins that are physiologically relevant to the drug's mode of action, including an operonic cluster of five genes encoding type II fatty acid synthase enzymes and *fbpC*, which encodes trehalose dimycolyl transferase. Insights gained from this approach may define new drug targets and suggest new methods for identifying compounds that inhibit those targets. In addition to the alternation in gene expression patterns related to the drug's mode of action, drugs can induce changes in genes related to stress responses that are linked to the toxic consequences of the drug. Each type of compound often generates a signature pattern of gene expression. A database populated with these signature profiles can serve as a guide to elucidate the potential mode of action as well as side effects of uncharacterized compounds.

Another successful application of DNA microarray techniques in medical microbiology is the determination of antimicrobial resistance by simultaneously detecting a panel of drug resistance-related mutations in microbial genomes (Call et al., 2003; Crameri et al., 2007; Hager, 2006; Perreten et al., 2005; Zhu et al., 2007a; Zhu et al., 2007b). The emergence of multidrug-resistant tuberculosis, extensively drug-resistant tuberculosis, and time-consuming phenotypic antimycobacterial susceptibility procedures has stimulated the pursuit of microarray platforms in antituberculosis drug resistance determinations. Oligonucleotide based DNA arrays have been used for parallel species identification and rifampin resistance-related mutations in mycobacteria (Troesch et al., 1999) and, more specifically, for the detection of *M. tuberculosis* strains that are resistant to rifampin (Yue et al., 2004) or isoniazid, kanamycin, streptomycin, pyrazinamide, and ethambutol (Gegia et al., 2008). Oligonucleotide microarrays were developed to analyze and identify drug-resistant *M. tuberculosis* strains, and it was found that the results were comparable with those of standard antimicrobial susceptibility testing (Strizhkov et al., 2000; Mikhailovich et al., 2001). A low-cost and -density DNA microarray was designed to detect mutations that confer isoniazid and rifampin resistance in *M. tuberculosis* isolates. The low-cost and -density array protocol takes 45 min after PCR amplification, with only minimal laboratory equipment required (Aragon et al., 2006). Antonova and colleagues developed a method for the detection and identification of mutations in the *M. tuberculosis* genome determining resistance to fluoroquinolones by hybridization on biological microchips (Antonova et al., 2008). A recently developed QIAplex system combines a novel multiplex PCR amplification and suspension bead array identification for the simultaneous detection of 24 *M. tuberculosis* gene mutations responsible for resistance to isoniazid, rifampin, streptomycin, and ethambutol (Gegia et al., 2008).

Microarray-based techniques face several application challenges to determine antimicrobial resistance in the clinical setting. First, genomes of some pathogens continue to mutate under natural and therapeutic selective pressures, which is well demonstrated by HIV-1. An Affymetrix microarray was developed to provide HIV-1 antiretroviral-drug-resistant profiles (Kozal et al., 1996; Vahey et al., 1999; Wilson et al., 2000). The product was discontinued due to rapidly emerging HIV-1 genome mutations. The company now has a comprehensive, high-density microarray available to identify every mutation in resistance-related HIV-1 genomes. Second, molecular mechanisms for many antimicrobial drug resistances remain to be discovered while novel resis-

tance genes and mutations continue to emerge. It takes considerable time and effort to decipher all of the resistance-related mutations and transfer the basic science findings to clinical applications. For *M. tuberculosis*, until such knowledge is available, the currently used phenotypic methods for identifying resistance will continue to play an invaluable role in optimizing the therapy of persons with tuberculosis.

Staphylococcus aureus, including methicillin-resistant *S. aureus* (MRSA), is an important pathogen in hospitals and, increasingly, in communities around the world. Advanced laboratory techniques, including diagnostic microarray analysis, have been sought to rapidly identify staphylococcal isolates and determine antimicrobial susceptibility patterns. DNA microarray analyses of large samples of clinically characterized community-acquired MRSA strains have been reported, which provide broad insights into evolution, pathogenesis, and disease emergence (Koessler et al., 2006; Scherl et al., 2006). DNA microarrays based on the array-tube platform (ClonDiag Chip Technologies, Jena, Germany) have been used for characterizing and genotyping staphylococcal DNA, including their relevant resistance determinants and virulence factors (Monecke et al., 2007; Monecke and Ehricht, 2005; Monecke et al., 2006). Microarrays provide a valuable epidemiological tool for the detailed characterization of MRSA isolates and comparison of strains at a global level (Monecke et al., 2007).

Analyses of microbial evolution and epidemiology

DNA microarrays can be used to explore the variability in genetic content and in gene expression profiles within a natural population of the same or related species and between the ancestor and the descendents. As a result, it provides very rich information on the molecular basis of microbial diversity, evolution and epidemiology. Genomes within the species of *M. tuberculosis* have been compared with a high density oligonucleotide microarray to detect small-scale genomic deletions among 19 clinically and epidemiologically well-characterized isolates (Kato-Maeda et al., 2001).

This study reveals that deletions are likely to contain ancestral genes whose functions are no longer essential for the organism's survival, whereas genes that are never deleted constitute the minimal mycobacterial genome. As the amount of genomic deletion increased, the likelihood that the bacteria will cause pulmonary cavitation decreased, suggesting that the accumulation of mutations tends to diminish their pathogenicity.

CONCLUSION

In conclusion, applications of DNA microarrays for gene expression profiling experiments between two samples appear to be relatively reliable. The array technology, however, cannot give a reasonable estimation of the actual amount of mRNA. The measurement of relative abundance of particular mRNA species within the same sample needs to be further tested and improved.

Currently, both oligonucleotide and PCR product-based arrays are used for the study of bacterial species. Whether one format will prevail in the future will largely depend on robustness, feasibility (cost and availability of technology) and purpose of the experiments. For example, short oligonucleotide arrays may not be suitable for comparative genomic studies for organisms that are not closely related. Additionally, an individual array could be made of both oligonucleotides and PCR products.

The use of DNA microarrays as a tool for phylogenetic studies and strain identification merits attention. For many organisms, the 16S rRNA approach often fails to truly reflect their genetic potential. This gap can be bridged by comparative genomic methods with whole genome arrays in the absence of genome sequences. It is not difficult to envision the future construction of a DNA array that will contain unique sequences of 16S rRNA, 23S rRNA, and many key functional genes for most of the representative bacterial species. This array could be useful in food, medical, environmental, and agricultural applications.

With the increasing applications of DNA microarrays and generation of enormous quantities of data, the construction of a database and the linking of relevant functional information will be the next important phase of technology development. Centralization of genomic data, including DNA sequences and array results, will be very beneficial to the research community. The construction of EcoReg, EcoCyc and EcoSal web sites for *E. coli* is an excellent starting point and could serve as a model for other prokaryotic microorganisms.

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