Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Infection, Genetics and Evolution 9 (2009) 987–995

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Discussion

Characterization of microbial pathogens by DNA microarrays

Antoine Huyghe [a,b,*], Patrice Francois [a], Jacques Schrenzel [a]

[a] Genomic Research Laboratory, Infectious Diseases Service, University of Geneva Hospitals, Micheli-du-Crest 24, 1211 Geneva 14, Geneva, Switzerland
[b] Swiss Institute of Bioinformatics, Michel-Servet 1, 1211 Geneva 4, Geneva, Switzerland

ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 21 October 2008
Accepted 26 October 2008
Available online 17 November 2008

Keywords:
Microarray
Pathogen
Detection
CGH
Phylogeny
Bacteria

1. Introduction

Searching for the keyword 'microarray' in the NCBI database (June 2008 release) retrieved more than 25,000 hits, illustrating the popularity of this relatively recent technology that was first described in 1995 (Schena et al., 1995). Microarrays consist of multiple probes (nucleic acids, proteins, carbohydrates, antibodies) deposited or directly synthesized on a surface in an ordered fashion. This approach has several advantages over classical probe-based methods: high-throughput, parallelism, miniaturization and automation.

Conventional DNA microarrays consist of nucleic acid probes deposited on a planar glass surface. The surface is usually coated with chemically reactive groups (epoxy, poly-L-Lysine or aldehyde) to ensure efficient binding of nucleotidic probes on the surface. To assess the presence of target genes, nucleic acid samples are labeled, either chemically or by an enzymatic reaction. Labeled samples are then hybridized onto the array, and washed using different stringency buffers. The remaining signal resulting from specific interactions between probes and target nucleic acids is measured using a confocal microarray scanner. Only probes hybridized to a labeled target will yield signal, thus revealing the presence of the cognate nucleic acid motif in the sample.

High-density DNA microarrays have been used in a broad variety of applications such as transcriptomics, comparative genome hybridization (CGH), resequencing, drug discovery, microbial community characterization or single nucleotide polymorphism (SNP) analysis. The field of application depends primarily on the strategy and the marker genes used to design the probes. A variety of genes (virulence factors, phylogenetic markers, antibiotic resistance genes, etc.) have been employed on microbial diagnostic microarrays, depending on the question raised by the researcher.

Microarrays have thus been instrumental in the detection of known pathogens as well as in the discovery of novel infectious agents, such as SARS (Wang et al., 2003). Their performance has also been described in the detection of co-infecting agents during mixed infections (Lin et al., 2006, 2007). To the best of our knowledge, the utilization of microarrays is currently limited to research laboratories and no such technology is yet employed in routine clinical practice. In this review, we will inventory the proposed strategies for the detection of microbial pathogens.

2. Strategies and markers

2.1. Genome-oriented strategies

2.1.1. Whole-genome ORF arrays

A whole-genome ORF array contains probes recognizing the majority of the ORF content of one or several genomes. This approach is applicable to many different types of studies,

* Corresponding author.
E-mail address: antoine.huyghe@genomic.ch (A. Huyghe).

1567-1348/$ – see front matter
doi:10.1016/j.meegid.2008.10.016

explaining its popularity. Typically, this strategy is implemented for gene expression profiling studies (i.e. transcriptomics) to monitor the expression of mRNAs in the organism of interest under different growth conditions or during time-series analyses. Alternatively, whole-genome ORF arrays can be exploited for comparative studies, now relying on the hybridization of genomic DNA. Such comparative genome hybridization (CGH) studies can permit assessment of the genetic relatedness between a collection of organisms from the same genus and/or species, by evaluating at the genome scale the presence of various microevolution events (acquisition of genes by lateral transfer as well as deletions and rearrangements). Typically, the DNA of the reference genome is co-hybridized with the strain being characterized (Fig. 1A), in order to provide a positive control on each probe (Koessler et al., 2006). Comparison of log ratio values for all fluorescent signal intensities makes it possible to distinguish the presence/absence of genes in the studied strain (Kim et al., 2002). Whole-genome comparisons typically identify sets of core genes that are shared by a large majority of strains in the same species (Grasselli et al., 2008; Leonard et al., 2003; Lindsay et al., 2006), as well as accessory genes that are present in a subset of strains from the species that typically result from lateral gene acquisition. These differences can often be used to identify genes and/or genetic islands related to 'gain-of-function traits' in pathogenic strains.

A CGH study (Fitzgerald et al., 2001) that was carried out to investigate molecular population genetics on a panel of *Staphylococcus aureus* clinical isolates showed that about 22% of the genes are accessory. Most of the species-specific genes are located in chromosomal regions carrying genes involved in virulence or antibiotic resistance mechanisms. This study also underlined the importance of horizontal gene transfers in the evolution of *S. aureus* genome, notably in the acquisition and evolution of the

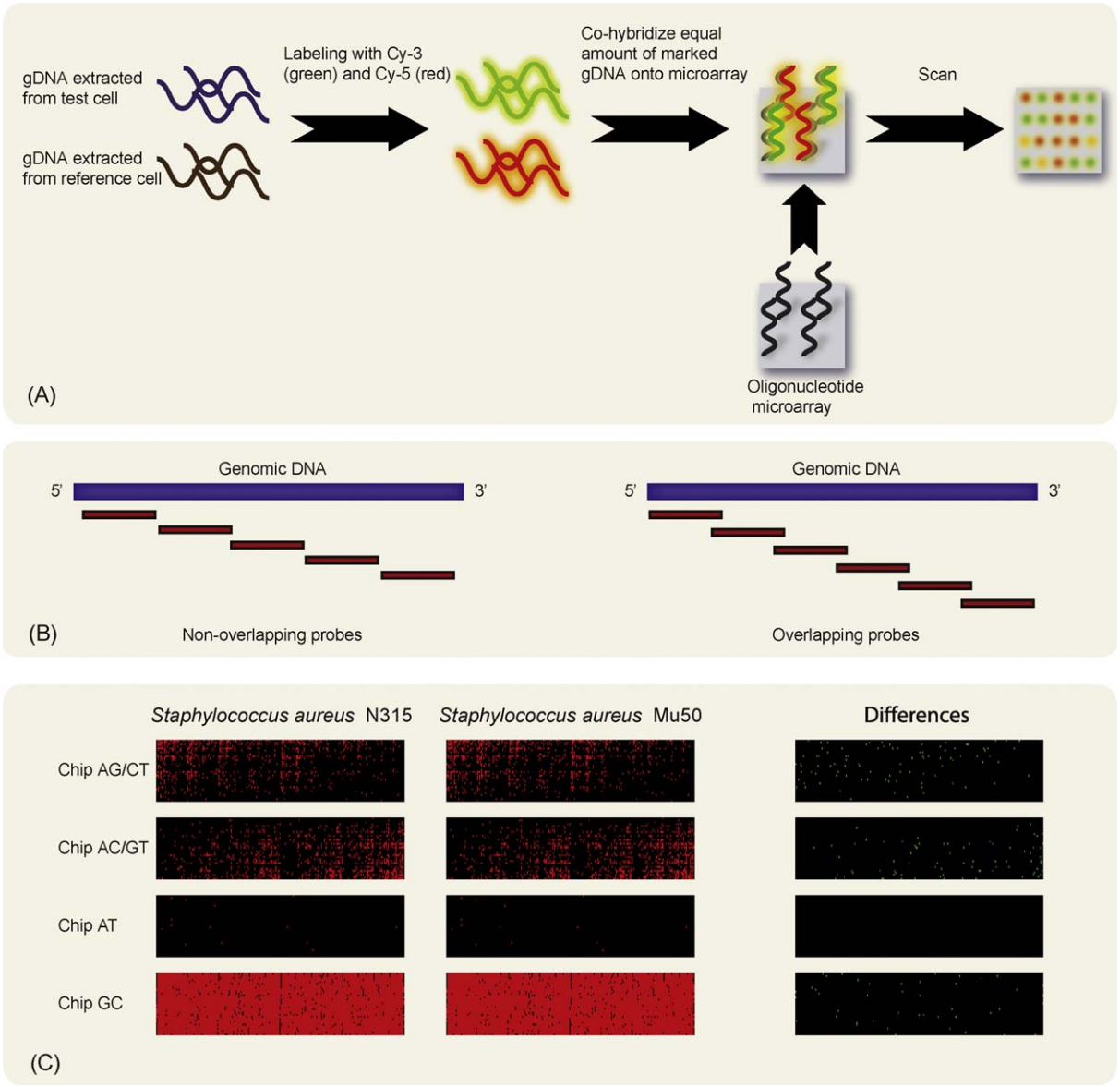

Fig. 1. (A) Principle of the comparative genomic hybridization (CGH). Equal amounts of genomic material appear in orange on the scanned array. (B) Probes disposition in a tiling array design. (C) Virtual hybridization of two methicillin-resistant *S. aureus* strains on NCHS chips and their differential hybridization patterns. This figure shows *in silico* hybridization (red) of the related *S. aureus* strains N315 and Mu50 (displaying decreased susceptibility to glycopeptides) on the four different types of NCHS chips (13-mers di-nucleotides). The differential pattern (green) represents probes that hybridize exclusively to either one of the two strains, yielding to a total of 216 informative probes for the four NCHS chips.

mecA gene responsible for methicillin resistance. Similarly, a CGH study focusing on the genomic characterization of community- and hospital-acquired methicillin-resistant *S. aureus* (Koessler et al., 2006) showed that this approach is well suited for discovering novel marker genes and chromosomal regions in a collection of clinical isolates. CGH is an ideal genomotyping tool with a better resolution than more typical molecular techniques such as characterizing variable numbers of tandem repeats (VNTR) or multilocus sequence typing (MLST).

2.1.2. Tiling arrays

With this strategy, probes are tiled along the entire genome length in an overlapping fashion or in close proximity (Fig. 1B). Although the number of probes required to cover a whole genome can rapidly become unwieldy, tiling arrays are conveniently used in resequencing and for single nucleotide polymorphisms (SNP) detection studies (Herring and Palsson, 2007; Kozal et al., 1996; Lin et al., 2006; Wong et al., 2004).

Use of resequencing microarrays allows generating sequences and determining SNPs to provide a way to track genetic variants, making this approach ideally suited for outbreaks surveillance. Typically, a resequencing microarray is composed of probes tiled on a sequence of interest with the central nucleotide permutated with all four possible combinations. The introduction of this central polymorphic site makes this approach ideal to define SNPs in a gene (Hu et al., 2008).

Wang et al. (2006) used this approach to identify influenza viruses involved in respiratory infections. This team developed a respiratory pathogen microarray including probes targeting partial sequences from hemagglutinin (HA), neuraminidase (NA) and matrix (M) genes of influenza A and B viruses. Using a random amplification protocol, this study demonstrated that the sequences obtained with the array were identical to conventional sequencing except for ambiguous base calls, and enabled comparison of the sequence of the isolated virus to previously sequenced reference viruses. Resequencing microarrays not only permit characterization of respiratory viruses at the species level (pattern recognition), but also at the strain level, which makes this a powerful tool for epidemiologic surveillance.

2.2. Metagenomic strategies

2.2.1. rRNA gene arrays

Conventional molecular diagnosis tests designed to identify bacterial strains usually rely on the amplification of the universal rRNA genes. These genes are particularly relevant targets for identification purposes for several reasons: (i) universality among bacteria, (ii) a combination of both conserved and divergent sequence regions, (iii) abundance in cells (frequently present as multi-copy genes and very highly transcribed, leading to high rRNA concentration), (iv) and the fact that lateral transfer of rRNA genes is extremely rare, explaining why they can be used as phylogenetic markers (Claridge et al., 1997). The resulting amplicons can be analyzed by various techniques such as denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (T-RFLP) or sequencing. Unsurprisingly, a plethora of microarray designs take advantage of the intrinsic potential of this gene as a phylogenetic marker to characterize microbial pathogens.

Among rRNA molecules, the bacterial 16S rRNA is particularly popular and benefits from a large array of public sequence databases (ARB (Ludwig et al., 2004), Greengenes (DeSantis et al., 2006) or RDP-II (Maidak et al., 2001) among others), making this gene particularly appealing for routine bacterial identification. A variety of microarrays using probes targeting the 16S rRNA gene have been successfully validated for the identification of bacteria from clinical samples (Francois et al., 2006; Liu et al., 2005; Zhu et al., 2007) or environmental niches (Neufeld et al., 2006; Brodie et al., 2006; Loy et al., 2005). A diagnostic array based on 16S rRNA was developed to detect 23 bacterial bloodstream pathogens (Wiesinger-Mayr et al., 2007) using 74 short (20–30 bp) oligonucleotidic probes. This team showed that the approach was able to detect and identify closely related species in a range of 10^1–10^3 bacteria from pure cultures, and 10^1–10^5 bacteria per ml of spiked blood. The observed performance was markedly dependant on the bacterial species. Although all probes did not show species-specific signals, a machine learning algorithm (*k*-nearest neighbor) was used to classify hybridization patterns by similarity to hybridizations of known organisms. Similarly, Francois et al. (2006) used classification algorithms on a Ta_2O_5-coated microarray coated with 19-mer signature oligonucleotides targeting the 5′-end of 16S rRNA genes of human pathogenic bacteria. In an approach unique to this study, the authors hybridized labeled RNA directly coupled to fluorescent dye to avoid biases related to the amplification method. In this study, classification of hybridization patterns was performed using decision tree and artificial neural networks, most recently achieving a confidence >96% in bacterial identification. Interestingly, the authors of both studies did not record any cross-hybridization due to the presence of human nucleic acid but highlighted the need for sophisticated analytical methods instead of simply monitoring signal levels of cognate probes.

However, the main limitation of the 16S rRNA gene relies in its high degree of conservation. In some cases, it can be impossible to design species-specific probes. A notable example exists in the *Enterobacteriaceae* family. To overcome this problem, alternative regions within the ribosomal operon have been proposed, such as the 5S and 23S (Hong et al., 2004) as well as the rDNA internal transcribed spacer (ITS) region (Leaw et al., 2007; Nübel et al., 2004). Various properties make the ITS region especially suitable for the identification of fungi: (i) this region is ubiquitous to all fungi, (ii) it is present in multiple copies, and (iii) ITS regions offer a higher level of variability compared to rRNA genes, thus making this region suitable for identification at the species level. In order to characterize fungal pathogens, a large variety of microarrays were developed on the basis of the ITS region with good reproducibility and specificity (Hsiao et al., 2005; Leinberger et al., 2005; Huang et al., 2006).

2.2.2. Functional gene arrays

Functional gene arrays (FGAs) are DNA microarrays containing probes that target a conserved region within a family of genes of interest, displaying specific functions. FGAs can be used to ensure a higher phylogenetic granularity when the degree of similarity between species/strains is too narrow to perform reliable identification using only ribosomal regions. Secondly, this approach appears particularly useful for assessing the presence and activity of key enzymes, antibiotics resistance genes or virulence factors from microbial samples, which may be more important, in some instances, than identifying species with other phylogenetic criteria. Primarily used to study the metabolic potential of environmental bacterial samples (e.g. biodegradation microbial communities (Rhee et al., 2004), biogeochemical processes (He et al., 2007) or nitrogen cycling (Tiquia et al., 2004)), this approach successfully detected genes involved in virulence and antibiotic resistance mechanisms (Jaing et al., 2008; Sergeev et al., 2006; Volokhov et al., 2002). For instance, Sergeev et al. (2006) implemented a FGA testing for the presence of 18 virulence factor genes in the subtype *Bacillus* group and they showed that this strategy can indeed provide strain typing information in addition to the inventory of virulence factors. A similar strategy was also described for different bacteria of clinical

importance (Saunders et al., 2004; Perreten et al., 2005). The description of a high density arrays targeting a comprehensive collection of genes involved in virulence and antibiotics resistance mechanisms was recently reported (Jaing et al., 2008). For the first generation array, this team identified 1245 target sequences representing 160 virulence and antibiotics resistance gene families derived from 2 *Escherichia coli* strains (CFT073 and K12), *E. faecalis* and *S. aureus*. Two important observations emerge from this study. Firstly, when using proper whole-genome amplification, this array was able to detect low quantities (3.1 fg) of purified DNA spiked in aerosol samples. Secondly, this FGA proved successful in detecting orthologs of different species in the same genus. Thus, while providing strain-level identification, this array could also be used as a tool to describe virulence factors in environmental samples containing yet un-sequenced organisms.

2.2.3. Phylogenetic arrays

Characterizing the content of complex microbial samples is usually achieved by sequencing cloned PCR products, after amplification of the small rRNA subunit (16S rRNA) using "universal" primers. Still considered the 'gold standard method', this approach remains laborious, costly and the number of clones required to obtain a comprehensive inventory of the bacterial content of a sample can be tremendously large. Note also that the first amplification step introduces – by definition – a bias between the original sample composition and the final amplification products. Taking advantage of the high parallelism property of microarrays, phylogenetic microarrays have been proposed as a convenient alternative to study complex bacterial flora. The underlying concept is to design probes recognizing sequence signatures specific for each node of the phylogenetic tree, extending the scope of oligoarrays to the whole bacterial kingdom. Many phylogenetic markers can be used for the design, such as groEL (Wertz et al., 2003; Wong and Chow, 2002), gyrA/gyrB (Antwerpen et al., 2007), rpoB (Drancourt et al., 2004; Troesch et al., 1999) or rpsA (Martens et al., 2007), but once again the rRNA gene remains the preferred candidate because of the number of available databases dedicated to this marker (see Section 2.2.1). Several teams developed and validated this approach (Palmer et al., 2006; Huyghe et al., 2008; Brodie et al., 2007), proving its efficiency in monitoring the contents of complex bacterial mixtures in a quantitative (fractional abundance $<0.1\%$) and qualitative fashion. Our group recently published a validation of a bacterial phylogenetic microarray (Huyghe et al., 2008) composed of oligonucleotidic probes targeting the 16S rRNA gene. Microarray design was performed by selecting about 9500 short oligonucleotides (25-mers, poly(T)-tailed to reach an overall length of 60 nucleotides) specific to nodes matching the different levels of the whole bacterial phylogenetic tree (Fig. 2). As part of the validation process, this array was used to characterize the bacterial gingival flora of two healthy subjects and results showed good agreement with previous cloning sequencing studies. Compared to cloning libraries, we also noted that phylogenetic microarrays revealed a broader bacterial diversity, a finding already reported by similar studies (Brodie et al., 2007; DeSantis et al., 2007), which could be explained by cloning bias, or by the paucity of clones sequenced. We developed this strategy in order to elucidate the etiological agent(s) of noma (Baratti-Mayer et al., 2003), a gangrenous disease affecting children and leading to the destruction of hard and soft tissues of the face. Our preliminary results (manuscript in preparation) suggest an imbalance in bacterial composition when comparing healthy children and noma patients, with a broader bacterial diversity in unaffected children. In contrast, *Peptostreptococcus* spp., *Prevotella* spp. and *Spirochaetaceae* are dominant phylotypes in noma lesions. Briefly, this study illustrates the usefulness of phylogenetic strategies in the understanding of unknown etiologies when a microbial origin is suspected.

Applying an rRNA phylogenetic microarray to monitor the temporal and geographical dynamics of bacterial populations in urban aerosols, Brodie et al. (2007) detected a surprising variety of human and animal pathogenic bacteria in the air of two American cities (Austin and San Antonio). The causative agents of glanders and melioidosis, *Burkholderia mallei* and *B. pseudomallei*, respectively, were regularly detected during the study; similarly, different pathogens such as *Campylobacteraceae*, *Clostridium botulinum*, *Helicobacter* and tick-borne *Rickettsia* were also detected in aerosol samples. This study showed that phylogenetic oligoarrays can be easily used to characterize bacterial pathogens from a dense background of non-pathogenic bacteria and could, for example, be exploited as part of a bio-surveillance initiative.

2.3. Universal pathogen diagnostic microarrays

In comparison to bacteria, the absence of ubiquitous gene targets makes the design of viral microarrays much more complex. An interesting approach was proposed by Wang et al. (2002) using a pan-viral microarray composed of approximately 1600 probes representing the most highly conserved 70-mers from 140 animal and human viruses. Probes were selected from each virus in order to maximize potential cross-hybridization to conserved regions and allowing detection of non-sequenced or unknown viruses. This strategy was combined with random PCR amplification, i.e. without use of sequence-specific primers. It proved very useful in identifying a previously uncharacterized coronavirus as the causative agent of an outbreak of severe acute respiratory syndrome (SARS) in 2003 (Wang et al., 2003). An unknown virus recovered from a SARS patient was cultured and hybridized on this pan-viral array. Probes with the highest hybridization intensities suggested that the virus belonged to – but was likely a new member of – the coronavirus family. Another original feature of this study was that the hybridized material was again retrieved from the array, cloned and sequenced for further characterization of this novel virus, highlighting the potential of microarrays as preparative molecular biology tools. In a previous study (Wang et al., 2002), the same team demonstrated that this approach, besides being able to distinguish between related viral serotypes, seems well adapted for detecting previously uncharacterized viruses. Indeed, maximizing potential cross-hybridization in conserved regions within the same viral genus allows identification of unsequenced viruses.

To date, the most comprehensive multi-microbial detection microarray was proposed by Palacios et al. (2007). This team developed a panmicrobial microarray comprising oligonucleotide probes targeting 1710 virus species and 135 bacterial, 73 fungal and 63 parasite genera. In addition, a few hundred probes recognizing host immune response genes were added to the design to potentially assess the host response. Unsurprisingly, the ribosomal RNA region was selected as a target marker for the detection of bacterial (16S), fungal (18S) and parasitic (18S) pathogens, while viral probes were selected from highly conserved regions. Using viral RNA extracted from cultured cells, detection limits of this microarray ranged from 1900 RNA copies for RNA viruses to 10,000 RNA copies for DNA viruses. The performance of the array was tested on clinical samples from patients suffering from various bacterial and viral diseases, and results correlated well with determinations performed by using classical methods. This panmicrobial approach has many interesting aspects, especially the possibility to identify unexpected pathogens. For example, the authors identified *Plasmodium falciparum* (the causative agent of malaria) in a patient formerly suspected of viral hemorrhagic-like disease.

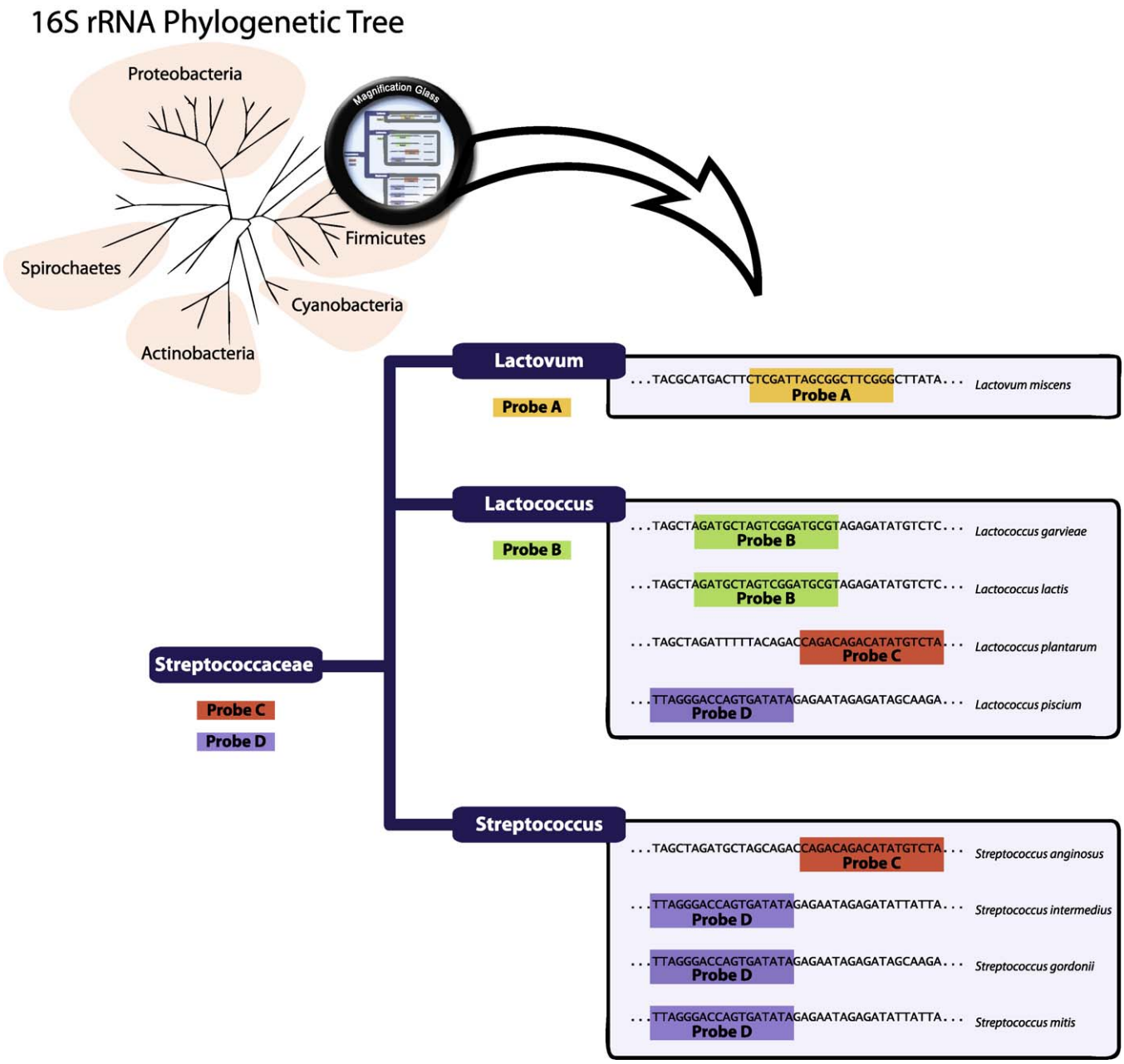

Fig. 2. Schematic representation of the probe selection process characterizing the 16S rRNA phylogenetic microarray as proposed by Huyghe et al. (2008). Candidate probes are compared to a 16S rRNA sequences database and assigned to the most distal common node. For example, probe D, which is common to the *Streptococcus* and *Lactococcus* genera, is assigned to the *Streptococcaceae* family level. In contrast, probe B is specific to some *Lactococcus* species, and it is therefore assigned to the genus level.

The design of the broadest panmicrobial diagnosis microarray involves the extensive knowledge of pathogen sequences, meaning that classical probe designs relying on prior knowledge of nucleotide sequences are limited by the number of known sequenced species and the quality of sequencing. To overcome this limitation we proposed a non-cognate approach relying on a probe design scheme that enables targeting multiple organisms without having prior knowledge of their genomic content. This experimental strategy, Non-cognate hybridization system (NCHS; patent WO/2000/075377; manuscript in preparation), is based on the utilization of two microarrays containing short probes (Fig. 1C) providing all combinations of only two nucleotides (i.e. A and C for the first array; A and G for the second array). Hybridization of genomic DNA from bacterial strains using this strategy results in a strain-specific probe pattern allowing direct identification of bacterial contents in samples. Though pattern identification relies on sequence databases, pattern recognition algorithms (such as self-organizing maps) can be used to identify unknown pathogens by homology to closely related and known organisms. However, due to the high variation in the GC content of capture elements, experimental conditions have to be tailored to limit potential bias during hybridization. The utilization of chemical compounds such as formamide (Sarkar et al., 1990), betaine (Henke et al., 1997) or tetramethylammonium chloride (Sorg et al., 1991) during hybridization or washing steps generally limit effect related to heterogeneity in melting temperatures of probes. Although this approach needs extensive enhancement and validation, it should prove efficient in detecting a broad variety of microbial pathogens.

3. General considerations for probe selection

The key point for setting up oligonucleotide microarray studies is the selection of an adequate set of probes. Ideally, three points must be considered during the probe selection process. First, all probes must be highly specific for the target gene while avoiding cross-hybridization with non-target sequences (specificity). Secondly, probes must display a high sensitivity in order to detect low abundant target sequences from a sample. Finally, the set of probes must exhibit homogenous thermodynamic properties ensuring similar hybridization behavior.

Oligonucleotide length plays a crucial role in the probe tolerance to mismatches towards its target. Short probes (25–35 nt) tend to be more adapted to identify mismatched position**s**, and are thus probably more suitable for phylogenetic microarrays. However, longer probes display better sensitivity but weaker specificity (Relogio et al., 2002). A good strategy to increase sensitivity relies on the use of spacers, such as thymidine tails, on short oligonucleotides. Increasing the length of short probes reduces steric hindrance by pushing the probe away from the array surface (Peplies et al., 2003).

Presence of mismatches at the central position of short oligonucleotides tends to affect probe-target duplex stability. For longer oligonucleotides, mismatches in the distal region (solution end) have less impact on the probe-target duplex stability than mismatches on the proximal region (surface end) of the probe (Hughes et al., 2001).

Both PCR products (cDNA) and oligonucleotides can be used as probes for microarray fabrication. Although PCR is relatively simple to implement in an average laboratory, oligonucleotide probes are more reliable in terms of sensitivity and specificity (Li et al., 2002; Kothapalli et al., 2002; Barczak et al., 2003), and provide a flexible design.

From a structural point of view, probe-target hybridization is influenced by the formation of inter- and/or intra-molecular secondary structures (stem loops, helices, dimerization) and by the affinity of the probe to bind to a target; which follow thermodynamic predictions. Various free tools, such as Hyther (Bommarito et al., 2000), Mfold (Zuker, 2003), or RNAfold (Hofacker and Stadler, 2006), are available for the estimation of thermodynamic properties of probes and probe-target duplexes, including prediction of Gibbs free energies (ΔG) and melting temperatures (T_m). However these tools rely on nearest-neighbor models and have been described with experimental observations performed in solution and may not reflect the actual properties of probes attached on a solid surface. This difference was recently illustrated by Pozhitkov et al. (2006) who observed a poor statistical correlation between prediction of Gibbs free energies and signal intensities of oligoarray probes targeting eukaryotic rRNA genes.

In summary, thermodynamic evaluation of oligonucleotide properties helps for optimizing hybridization sensitivity and specificity; nonetheless, careful wet-lab testing is required to evaluate hybridization properties of oligonucleotide probes before considering their use in other studies.

4. Sample preparation

Several caveats should be considered when discussing potential application of diagnostic arrays for routine bacterial identification. The risk of false-negative results is real and should be minimized. Most published microarray methods use amplification strategies prior to hybridization that are based on the polymerase chain reaction (Saiki et al., 1988; Mullis et al., 1986), a proven and sensitive technique. However, PCR is susceptible to numerous inhibitory compounds commonly present in clinical materials (Monteiro et al., 1997; Fredricks and Relman, 1998; Longo et al., 1990); fastidious sample preparation is thus required to obtain robust detection (Klein et al., 1997). In addition, some microorganisms potentially responsible for infections harbor specific envelopes or cell-walls which are either difficult to disrupt or further release inhibitors of enzymatic reactions. All these parameters should be considered carefully depending on the intended application and the types of samples to be analyzed, which both affect the various steps needed for detection or identification purposes.

Labeled nucleic acids consist of either DNA or RNA. Most of the time, nucleic acids require enzymatic reactions to be performed prior to hybridization, to either amplify and/or label the products. Characterization or genotyping microarrays typically rely on randomly amplified DNA fragments, often generated by the Klenow fragment (Koessler et al., 2006; Charbonnier et al., 2005) or a phage polymerase. The latter allows converting minute amounts of DNA to μg of hybridizable products (Pinard et al., 2006). Expression microarrays use either labeled RNA (Francois et al., 2006) or products of these RNAs obtained by reverse-transcription (Scherl et al., 2006). Recently, alternative RNA amplification strategies have been used, allowing for a reduction of the amount of starting cell numbers (Francois et al., 2007; La et al., 2007), an important issue in many biological applications.

At least two different strategies are currently available to fluorescently label nucleic acids, either directly or indirectly. The direct incorporation uses nucleotide derivatives that are directly coupled to fluorescent molecules (Scherl et al., 2006). An enzymatic amplification enables the incorporation of such residues and yields to fluorescently-labeled nucleic acids that can be resolved by hybridization on the array. Indirect labeling is a two-step reaction (Schroeder et al., 2002): the first step consists of incorporating nucleotides containing small chemically reactive groups, generally aminoallyl dUTP. Subsequently, activated fluorescent dyes are coupled (generally *N*-hydroxysuccinimidyl ester cyanine, such as Cy-3 and Cy-5) yielding labeled nucleic acids. This two-step strategy reduces the risk of biases introduced during the enzymatic incorporation of modified nucleotides, mostly due to the steric effects related to the presence of large aromatic fluorescent molecules.

While the use of fluorescent dyes is well established, alternative non-fluorescent labeling methods are available. An affordable alternative is to couple biotin-derivatives to target molecules, and then capture them with a streptavidin-conjugate enzyme (e.g. horseradish peroxidase HRP – streptavidin). Afterwards, numerous HRP chromogenic (e.g. tetramethylbenzidine) or chemiluminescent substrates can be used to detect hybridized targets. Resonance light scattering (RLS) can also be used as a more sensitive alternative to fluorescent labeling methods. When a gold (or silver) nanoparticle is illuminated with a white light beam, scattering produces an emitted light characterized by a defined wavelength. RLS can be exploited as a labeling method after hybridization of biotinylated targets and incubation with nanoparticles coupled with streptavidin. The procedure was tested against conventional fluorescence methods (Francois et al., 2003) to directly label and detect bacterial RNA without prior enzymatic amplification, thus limiting potential amplification bias and processing time. Compared to fluorescent labeling, RLS produces a consistently higher signal-to-noise ratio while providing a gain of about 50-fold in detection sensitivity.

5. Conclusion

DNA microarray technology, consisting of the hybridization of nucleic acid fragments (either from chemical or enzymatic

Table 1
Comparison of microarrays used for microbial pathogens characterization.

Array type	Target	Prior knowledge of sequence	Applicable to uncharacterized organisms	Highest resolution	Sensitivity	Limits
Whole-genome arrays	ORF (and inter-ORF)	Yes	Yes	Strains	–	Organism specific
Functional gene arrays	Family of functional genes	Yes	Yes	Species	3.1 fg (Jaing et al., 2008) • 10^1 bacteria (Wiesinger-Mayr et al., 2007)	Limited to specific functional families
Conventional rRNA arrays	rRNA gene	Yes	Yes	Species	• 10^1 bacteria (Wiesinger-Mayr et al., 2007) • 2.5–5.0 fmole of *S. aureus* 16S (Francois et al., 2006)	Design of species-discriminating probes can be tricky
Phylogenetic microarrays	rRNA gene or housekeeping genes	Yes	Yes	Species	0.1% of total community (Palmer et al., 2006)	Limited to phylogenetic studies
Tiling arrays	Whole genome	Yes	Yes	Strains-SNP	–	Organism specific
Universal microarrays						
Palacios et al.	rRNA gene and functionally related sequences	Yes	Yes	Species	• RNA viruses: 1900 RNA copies • DNA viruses: 10,000 RNA copies	Design of species-discriminating rRNA probes can be tricky
NCHS	Whole genome	No	Yes	Strains	To be determined	Inhomogeneous GC content requires use of additives

synthesis) immobilized onto a solid surface with the nucleic acid of a test sample, was first reported at the end of the 1990s (Schena et al., 1995). Constant improvements have been made regarding on properties of chip surfaces, nucleic acid density, the design of capture element, the dyes used to label the samples as well as in the sensitivity of detection devices. The format of DNA microarrays appears particularly adapted to the identification, characterization or genotyping of isolated microbial pathogens as well as to more clinical questions such as the assessment of virulence or antibiotic resistance genes. However, direct analysis of nucleic acid is generally not possible and the lack of sensitivity of the technology requires the use of amplification methods, introducing some potential biases in the content of the sample. To date, no commercial microbial diagnostic microarrays, approved by the food and drug administrations, are available on the clinical diagnostics market.

One of the major challenges in that field derives from the nature of the sample. Biological fluids are complex medium potentially containing pathogenic organisms (often rare) in a community of commensal organisms and in the presence of variable amounts of host material. In such cases, amplification of the pathogenic material is essential while the detection strategy must avoid amplified host nucleic acids. In addition, characterization and identification of co-infective agents represents another major challenge.

The high parallelism of microarray platforms makes it possible to address thousands of questions during a single experiment; they appear therefore particularly suited for bacterial detection or identification. Furthermore, isolated microorganisms can be further characterized using high resolution genotyping (Quiñones et al., 2007; Zhang et al., 2006), by providing an extensive documentation of virulence factors (Lindsay et al., 2006; Saunders et al., 2004) or for specific epidemiological survey purposes (Garaizar et al., 2006). Note that the depth or precision of data collected is generally dependent on the number of capture elements present on the surface of the microarray. This number varies from hundreds (Saunders et al., 2004) for virulence factors to hundreds of thousands for a resolution at the nucleotide level (Zhang et al., 2006). For specific applications, a resolution at the nucleotide level is also achievable using more elaborated strategies such as padlock probes (Szemes et al., 2005).

Generally, the question to be answered will guide the choice of both the approach and the markers (see Table 1) to assess identification. For example, rRNA genes are undoubtedly the most common gene marker used in microbial detection microarrays. However, this phylogenetic marker is not always adapted for species determination, due to: (i) its high conservation between certain closely related species, and (ii) lack of accurate thermodynamic properties to assess quality of probes targeting rRNA genes (Pozhitkov et al., 2006). Alternative phylogenetic markers may be preferable in that specific situation. In addition, the design of microarrays based on cognate approaches is entirely dependent on the quality of sequence databases available to researchers. To date, a small fraction of the microbial world has been sequenced and characterization of unknown pathogens using microarray involves making assumptions from sequenced genomes. These different challenges appear surmountable using adapted design strategies, technical procedures and specific analytical solutions.

Over the past year, high throughput DNA sequencing technology has become more accessible to the scientific community and could, in the very near future, become an alternative to microbial diagnostics microarrays. However, microarrays will not necessarily be put aside as they could, for example, be exploited as a preparative platform to capture nucleic acids (Porreca et al., 2007; Wang et al., 2003). Clinical microbiology is rapidly evolving and molecular methods are now extensively challenging conventional microbiology techniques. The diagnostic field will benefit from this evolution as microarray technology appears to be the ideal tool to assess the diversity of the microbial world as well as to improve our knowledge of host–pathogen interactions.

Acknowledgements

The authors' efforts in preparing this review were supported by the COST C05.0103 (JS) (Action B28: Array Technologies for BSL3 and BSL4 Pathogens–Workgroup 1) and by grants from the Swiss

National Science Foundation 3100A0-116075/1 (PF) and 3100A0-112370/1 (JS). AH is supported by the Geneva Study Group on Noma (GESNOMA), a research group funded by the Hirzel Foundation. Many thanks to Dr Katrine Whiteson for careful reading of the manuscript.

References

Antwerpen, M.H., Schellhase, M., Ehrentreich-Förster, E., Bier, F., Witte, W., Nübel, U., 2007. DNA microarray for detection of antibiotic resistance determinants in Bacillus anthracis and closely related Bacillus cereus. Mol. Cell. Probes 21, 152–160.

Baratti-Mayer, D., Pittet, B., Montandon, D., Bolivar, I., Bornand, J.E., Hugonnet, S., Jaquinet, A., Schrenzel, J., Pittet, D., 2003. Noma: an "infectious" disease of unknown aetiology. Lancet Infect. Dis. 3, 419–431.

Barczak, A., Rodriguez, M.W., Hanspers, K., Koth, L.L., Tai, Y.C., Bolstad, B.M., Speed, T.P., Erle, D.J., 2003. Spotted long oligonucleotide arrays for human gene expression analysis. Genome Res. 13, 1775–1785.

Bommarito, S., Peyret, N., SantaLucia, J., 2000. Thermodynamic parameters for DNA sequences with dangling ends. Nucleic Acids Res. 28, 1929–1934.

Brodie, E.L., DeSantis, T.Z., Joyner, D.C., Baek, S.M., Larsen, J.T., Andersen, G.L., Hazen, T.C., Richardson, P.M., Herman, D.J., Tokunaga, T.K., Wan, J.M., Firestone, M.K., 2006. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Appl. Environ. Microbiol. 72, 6288–6298.

Brodie, E.L., DeSantis, T.Z., Parker, J.P.M., Zubietta, I.X., Piceno, Y.M., Andersen, G.L., 2007. Urban aerosols harbor diverse and dynamic bacterial populations. Proc. Natl. Acad. Sci. 104, 299–304.

Charbonnier, Y., Gettler, B., François, P., Bento, M., Renzoni, A., Vaudaux, P., Schlegel, W., Schrenzel, J., 2005. A generic approach for the design of whole-genome oligoarrays, validated for genomotyping, deletion mapping and gene expression analysis on *Staphylococcus aureus*. BMC Genomics 6, 95.

Claridge, M.F., Dawah, H.A., Wilson, M.R., Association, S., 1997. Species: The Units of Biodiversity. Springer.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072.

DeSantis, T.Z., Brodie, E.L., Moberg, J.P., Zubieta, I.X., Piceno, Y.M., Andersen, G.L., 2007. High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb. Ecol. 53, 371–383.

Drancourt, M., Roux, V., Fournier, P.E., Raoult, D., 2004. rpoB gene sequence-based identification of aerobic Gram-positive cocci of the genera Streptococcus, Enterococcus, Gemella, Abiotrophia, and Granulicatella. J. Clin. Microbiol. 42, 497–504.

Fitzgerald, J.R., Sturdevant, D.E., Mackie, S.M., Gill, S.R., Musser, J.M., 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc. Natl. Acad. Sci. 98, 8821–8826.

Francois, P., Bento, M., Vaudaux, P., Schrenzel, J., 2003. Comparison of fluorescence and resonance light scattering for highly sensitive microarray detection of bacterial pathogens. J. Microbiol. Methods 55, 755–762.

Francois, P., Charbonnier, Y., Jacquet, J., Utinger, D., Bento, M., Lew, D., Kresbach, G.M., Ehrat, M., Schlegel, W., Schrenzel, J., 2006. Rapid bacterial identification using evanescent-waveguide oligonucleotide microarray classification. J. Microbiol. Methods 65, 390–403.

Francois, P., Garzoni, C., Bento, M., Schrenzel, J., 2007. Comparison of amplification methods for transcriptomic analyses of low abundance prokaryotic RNA sources. J. Microbiol. Methods 68, 385–391.

Fredricks, D.N., Relman, D.A., 1998. Improved amplification of microbial DNA from blood cultures by removal of the PCR inhibitor sodium polyanetholesulfonate. J. Clin. Microbiol. 36, 2810–2816.

Garaizar, J., Rementeria, A., Porwollik, S., 2006. DNA microarray technology: a new tool for the epidemiological typing of bacterial pathogens? FEMS Immunol. Med. Microbiol. 47, 178–189.

Grasselli, E., François, P., Gutacker, M., Gettler, B., Benagli, C., Convert, M., Boerlin, P., Schrenzel, J., Piffaretti, J.C., 2008. Evidence of horizontal gene transfer between human and animal commensal *Escherichia coli* strains identified by microarray. FEMS Immunol. Med. Microbiol 53 (3), 351–358.

He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., Huang, Z., Wu, W., Gu, B., Jardine, P., Criddle, C., Zhou, J., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME J. 1, 67–77.

Henke, W., Herdel, K., Jung, K., Schnorr, D., Loening, S.A., 1997. Betaine improves the PCR amplification of GC-rich DNA sequences. Nucleic Acids Res. 25, 3957–3958.

Herring, C.D., Palsson, B., 2007. An evaluation of comparative genome sequencing (CGS) by comparing two previously-sequenced bacterial genomes. BMC Genomics 8, 274.

Hofacker, I.L., Stadler, P.F., 2006. Memory efficient folding algorithms for circular RNA secondary structures. Bioinformatics (Oxford, England) 22, 1172–1176.

Hong, B.X., Jiang, L.F., Hu, Y.S., Fang, D.Y., Guo, H.Y., 2004. Application of oligonucleotide array technology for the rapid detection of pathogenic bacteria of foodborne infections. J. Microbiol. Methods 58, 403–411.

Hsiao, C.R., Huang, L., Bouchara, J.P., Barton, R., Li, H.C., Chang, T.C., 2005. Identification of medically important molds by an oligonucleotide array. J. Clin. Microbiol. 43, 3760–3768.

Hu, G., Liu, I., Sham, A., Stajich, J.E., Dietrich, F.S., Kronstad, J.W., 2008. Comparative hybridization reveals extensive genome variation in the AIDS-associated pathogen Cryptococcus neoformans. Genome Biol. 9, R41.

Huang, A., Li, J.W., Shen, Z.Q., Wang, X.W., Jin, M., 2006. High-throughput identification of clinical pathogenic fungi by hybridization to an oligonucleotide microarray. J. Clin. Microbiol. 44, 3299–3305.

Hughes, T.R., Mao, M., Jones, A.R., Burchard, J., Marton, M.J., Shannon, K.W., Lefkowitz, S.M., Ziman, M., Schelter, J.M., Meyer, M.R., Kobayashi, S., Davis, C., Dai, H., He, Y.D., Stephaniants, S.B., Cavet, G., Walker, W.L., West, A., Coffey, E., Shoemaker, D.D., Stoughton, R., Blanchard, A.P., Friend, S.H., Linsley, P.S., 2001. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. Nat. Biotechnol. 19, 342–347.

Huyghe, A., Francois, P., Charbonnier, Y., Tangomo-Bento, M., Bonetti, E.J., Paster, B.J., Bolivar, I., Baratti-Mayer, D., Pittet, D., Schrenzel, J., 2008. Novel microarray design strategy to study complex bacterial communities. Appl. Environ. Microbiol. 74, 1876–1885.

Jaing, C., Gardner, S., McLoughlin, K., Mulakken, N., Alegria-Hartman, M., Banda, P., Williams, P., Gu, P., Wagner, M., Manohar, C., Slezak, T., 2008. A functional gene array for detection of bacterial virulence elements. PLoS ONE 3, e2163.

Kim, C.C., Joyce, E.A., Chan, K., Falkow, S., 2002. Improved analytical methods for microarray-based genome-composition analysis. Genome Biol. 3 (11) RESEARCH0065.

Klein, A., Barsuk, R., Dagan, S., Nusbaum, O., Shouval, D., Galun, E., 1997. Comparison of methods for extraction of nucleic acid from hemolytic serum for PCR amplification of hepatitis B virus DNA sequences. J. Clin. Microbiol. 35, 1897–1899.

Koessler, T., Francois, P., Charbonnier, Y., Huyghe, A., Bento, M., Dharan, S., Renzi, G., Lew, D., Harbarth, S., Pittet, D., Schrenzel, J., 2006. Use of oligoarrays for characterization of community-onset methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 44, 1040–1048.

Kothapalli, R., Yoder, S.J., Mane, S., Loughran, T.P., 2002. Microarray results: how accurate are they? BMC Bioinform. 3, 22.

Kozal, M.J., Shah, N., Shen, N., Yang, R., Fucini, R., Merigan, T.C., Richman, D.D., Morris, D., Hubbell, E., Chee, M., Gingeras, T.R., 1996. Extensive polymorphisms observed in HIV-1 clade B protease gene using high-density oligonucleotide arrays. Nat. Med. 2, 753–759.

La, M.V., François, P., Rovery, C., Robineau, S., Barbry, P., Schrenzel, J., Raoult, D., Renesto, P., 2007. Development of a method for recovering rickettsial RNA from infected cells to analyze gene expression profiling of obligate intracellular bacteria. J. Microbiol. Methods 71, 292–297.

Leaw, S.N., Chang, H.C., Barton, R., Bouchara, J.P., Chang, T.C., 2007. Identification of medically important candida and non-Candida yeast species by an oligonucleotide array. J. Clin. Microbiol. 45, 2220–2229.

Leinberger, D.M., Schumacher, U., Autenrieth, I.B., Bachmann, T.T., 2005. Development of a DNA microarray for detection and identification of fungal pathogens involved in invasive mycoses. J. Clin. Microbiol. 43, 4943–4953.

Leonard, E.E., Takata, T., Blaser, M.J., Falkow, S., Tompkins, L.S., Gaynor, E.C., 2003. Use of an open-reading frame-specific Campylobacter jejuni DNA microarray as a new genotyping tool for studying epidemiologically related isolates. J. Infect. Dis. 187, 691–694.

Li, J., Pankratz, M., Johnson, J.A., 2002. Differential gene expression patterns revealed by oligonucleotide versus long cDNA arrays. Toxicol. Sci. 69, 383–390.

Lin, B., Malanoski, A.P., Wang, Z., Blaney, K.M., Ligler, A.G., Rowley, R.K., Hanson, E.H., von Rosenvinge, E., Ligler, F.S., Kusterbeck, A.W., Metzgar, D., Barrozo, C.P., Russell, K.L., Tibbetts, C., Schnur, J.M., Stenger, D.A., 2007. Application of broad-spectrum, sequence-based pathogen identification in an urban population. PLoS ONE 2, e419.

Lin, B., Wang, Z., Vora, G.J., Thornton, J.A., Schnur, J.M., Thach, D.C., Blaney, K.M., Ligler, A.G., Malanoski, A.P., Santiago, J., Walter, E.A., Agan, B.K., Metzgar, D., Seto, D., Daum, L.T., Kruzelock, R., Rowley, R.K., Hanson, E.H., Tibbetts, C., Stenger, D.A., 2006. Broad-spectrum respiratory tract pathogen identification using resequencing DNA microarrays. Genome Res. 16, 527–535.

Lindsay, J.A., Moore, C.E., Day, N.P., Peacock, S.J., Witney, A.A., Stabler, R.A., Husain, S.E., Butcher, P.D., Hinds, J., 2006. Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. J. Bacteriol. 188, 669–676.

Liu, Y., Han, J.X., Huang, H.Y., Zhu, B., 2005. Development and evaluation of 16S rDNA microarray for detecting bacterial pathogens in cerebrospinal fluid. Exp. Biol. Med. 230, 587–591.

Longo, M.C., Berninger, M.S., Hartley, J.L., 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 93, 125–128.

Loy, A., Schulz, C., Lücker, S., Schöpfer-Wendels, A., Stoecker, K., Baranyi, C., Lehner, A., Wagner, M., 2005. 16S rRNA gene-based oligonucleotide microarray for environmental monitoring of the betaproteobacterial order "Rhodocyclales". Appl. Environ. Microbiol. 71, 1373–1386.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., Konig, A., Liss, T., Lussmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A.,

Lenke, M., Ludwig, T., Bode, A., Schleifer, K.H., 2004. ARB: a software environment for sequence data. Nucleic Acids Res. 32, 1363–1371.
Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T., Saxman, P.R., Farris, R.J., Garrity, G.M., Olsen, G.J., Schmidt, T.M., Tiedje, J.M., 2001. The RDP-II (Ribosomal Database Project). Nucleic Acids Res. 29, 173–174.
Martens, M., Weidner, S., Linke, B., de Vos, P., Gillis, M., Willems, A., 2007. A prototype taxonomic microarray targeting the rpsA housekeeping gene permits species identification within the rhizobial genus Ensifer. Syst. Appl. Microbiol. 30, 390–400.
Monteiro, L., Bonnemaison, D., Vekris, A., Petry, K.G., Bonnet, J., Vidal, R., Cabrita, J., Mégraud, F., 1997. Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. J. Clin. Microbiol. 35, 995–998.
Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., Erlich, H., 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb. Symp. Quant. Biol. 51 (Pt 1), 263–273.
Neufeld, J.D., Mohn, W.W., de Lorenzo, V., 2006. Composition of microbial communities in hexachlorocyclohexane (HCH) contaminated soils from Spain revealed with a habitat-specific microarray. Environ. Microbiol. 8, 126–140.
Nübel, U., Schmidt, P.M., Reiss, E., Bier, F., Beyer, W., Naumann, D., 2004. Oligonucleotide microarray for identification of Bacillus anthracis based on intergenic transcribed spacers in ribosomal DNA. FEMS Microbiol. Lett. 240, 215–223.
Palacios, G., Quan, P.l., Jabado, O.J., Conlan, S., Hirschberg, D.L., Liu, Y., Zhai, J., Renwick, N., Hui, J., Hegyi, H., Grolla, A., Strong, J.E., Towner, J.S., Geisbert, T.W., Jahrling, P.B., Büchen-Osmond, C., Ellerbrok, H., Sanchez-Seco, M.P., Lussier, Y., Formenty, P., Nichol, M.S., Feldmann, H., Briese, T., Lipkin, W.I., 2007. Panmicrobial oligonucleotide array for diagnosis of infectious diseases. Emerg. Infect. Dis. 13, 73–81.
Palmer, C., Bik, E.M., Eisen, M.B., Eckburg, P.B., Sana, T.R., Wolber, P.K., Relman, D.A., Brown, P.O., 2006. Rapid quantitative profiling of complex microbial populations. Nucleic Acids Res. 34, e5.
Peplies, J., Glöckner, F.O., Amann, R., 2003. Optimization strategies for DNA microarray-based detection of bacteria with 16S rRNA-targeting oligonucleotide probes. Appl. Environ. Microbiol. 69, 1397–1407.
Perreten, V., Vorlet-Fawer, L., Slickers, P., Ehricht, R., Kuhnert, P., Frey, J., 2005. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. J. Clin. Microbiol. 43, 2291–2302.
Pinard, R., de Winter, A., Sarkis, G.J., Gerstein, M.B., Tartaro, K.R., Plant, R.N., Egholm, M., Rothberg, J.M., Leamon, J.H., 2006. Assessment of whole genome amplification-induced bias through high-throughput, massively parallel whole genome sequencing. BMC Genomics 7, 216.
Porreca, G.J., Zhang, K., Li, J.B., Xie, B., Austin, D., Vassallo, S.L., LeProust, E.M., Peck, B.J., Emig, C.J., Dahl, F., Gao, Y., Church, G.M., Shendure, J., 2007. Multiplex amplification of large sets of human exons. Nat. Methods 4, 931–936.
Pozhitkov, A., Noble, P.A., Domazet-Loso, T., Nolte, A.W., Sonnenberg, R., Staehler, P., Beier, M., Tautz, D., 2006. Tests of rRNA hybridization to microarrays suggest that hybridization characteristics of oligonucleotide probes for species discrimination cannot be predicted. Nucleic Acids Res. 34, e66.
Quiñones, B., Parker, C.T., Janda, J.M., Miller, W.G., Mandrell, R.E., 2007. Detection and genotyping of Arcobacter and Campylobacter isolates from retail chicken samples by use of DNA oligonucleotide arrays. Appl. Environ. Microbiol. 73, 3645–3655.
Relogio, A., Schwager, C., Richter, A., Ansorge, W., Valcarcel, J., 2002. Optimization of oligonucleotide-based DNA microarrays. Nucleic Acids Res. 30, e51.
Rhee, S.K., Liu, X., Wu, L., Chong, S.C., Wan, X., Zhou, J., 2004. Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Appl. Environ. Microbiol. 70, 4303–4317.
Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239, 487–491.
Sarkar, G., Kapelner, S., Sommer, S.S., 1990. Formamide can dramatically improve the specificity of PCR. Nucleic Acids Res. 18, 7465.
Saunders, N.A., Underwood, A., Kearns, A.M., Hallas, G., 2004. A virulence-associated gene microarray: a tool for investigation of the evolution and pathogenic potential of *Staphylococcus aureus*. Microbiology 150, 3763–3771.
Schena, M., Shalon, D., Davis, R.W., Brown, P.O., 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467–470.
Scherl, A., François, P., Charbonnier, Y., Deshusses, J.M., Koessler, T., Huyghe, A., Bento, M., Stahl-Zeng, J., Fischer, A., Masselot, A., Vaezzadeh, A., Gallé, F., Renzoni, A., Vaudaux, P., Lew, D., Zimmermann-Ivol, C.G., Binz, P.A., Sanchez, J.C., Hochstrasser, D.F., Schrenzel, J., 2006. Exploring glycopeptide-resistance in *Staphylococcus aureus*: a combined proteomics and transcriptomics approach for the identification of resistance-related markers. BMC Genomics 7, 296.
Schroeder, B.G., Peterson, L.M., Fleischmann, R.D., 2002. Improved quantitation and reproducibility in Mycobacterium tuberculosis DNA microarrays. J. Mol. Microbiol. Biotechnol. 4, 123–126.
Sergeev, N., Distler, M., Vargas, M., Chizhikov, V., Herold, K.E., Rasooly, A., 2006. Microarray analysis of Bacillus cereus group virulence factors. J. Microbiol. Methods 65, 488–502.
Sorg, U., Enczmann, J., Sorg, R., Wernet, P., 1991. Rapid non-radioactive TMACl hybridization protocol employing enzymatically labeled oligonucleotides. Nucleic Acids Res. 19, 4782.
Szemes, M., Bonants, P., de Weerdt, M., Baner, J., Landegren, U., Schoen, C.D., 2005. Diagnostic application of padlock probes—multiplex detection of plant pathogens using universal microarrays. Nucleic Acids Res. 33, e70.
Tiquia, S.M., Wu, L., Chong, S.C., Passovets, S., Xu, D., Xu, Y., Zhou, J., 2004. Evaluation of 50-mer oligonucleotide arrays for detecting microbial populations in environmental samples. BioTechniques 36, 664–670 672, 674.
Troesch, A., Nguyen, H., Miyada, C.G., Desvarenne, S., Gingeras, T.R., Kaplan, P.M., Cros, P., Mabilat, C., 1999. Mycobacterium species identification and rifampin resistance testing with high-density DNA probe arrays. J. Clin. Microbiol. 37, 49–55.
Volokhov, D., Rasooly, A., Chumakov, K., Chizhikov, V., 2002. Identification of listeria species by microarray-based assay. J. Clin. Microbiol. 40, 4720–4728.
Wang, D., Coscoy, L., Zylberberg, M., Avila, P.C., Boushey, H.A., Ganem, D., DeRisi, J.L., 2002. Microarray-based detection and genotyping of viral pathogens. Proc. Natl. Acad. Sci. 99, 15687–15692.
Wang, D., Urisman, A., Liu, Y.T., Springer, M., Ksiazek, T.G., Erdman, D.D., Mardis, E.R., Hickenbotham, M., Magrini, V., Eldred, J., Latreille, J.P., Wilson, R.K., Ganem, D., DeRisi, J.L., 2003. Viral discovery and sequence recovery using DNA microarrays. PLoS Biol. 1, e2.
Wang, Z., Daum, L.T., Vora, G.J., Metzgar, D., Walter, E.A., Canas, L.C., Malanoski, A.P., Lin, B., Stenger, D.A., 2006. Identifying influenza viruses with resequencing microarrays. Emerg. Infect. Dis. 12, 638–646.
Wertz, J.E., Goldstone, C., Gordon, D.M., Riley, M.A., 2003. A molecular phylogeny of enteric bacteria and implications for a bacterial species concept. J. Evol. Biol. 16, 1236–1248.
Wiesinger-Mayr, H., Vierlinger, K., Pichler, R., Kriegner, A., Hirschl, A., Presterl, E., Bodrossy, L., Noehammer, C., 2007. Identification of human pathogens isolated from blood using microarray hybridisation and signal pattern recognition. BMC Microbiol. 7, 78.
Wong, C.W., Albert, T.J., Vega, V.B., Norton, J.E., Cutler, D.J., Richmond, T.A., Stanton, L.W., Liu, E.T., Miller, L.D., 2004. Tracking the evolution of the SARS coronavirus using high-throughput, high-density resequencing arrays. Genome Res. 14, 398–405.
Wong, R.S.Y., Chow, A.W., 2002. Identification of enteric pathogens by heat shock protein 60 kDa (HSP60) gene sequences. FEMS Microbiol. Lett. 206, 107–113.
Zhang, W., Qi, W., Albert, T.J., Motiwala, A.S., Alland, D., Hyytia-Trees, E.K., Ribot, E.M., Fields, P.I., Whittam, T.S., Swaminathan, B., 2006. Probing genomic diversity and evolution of *Escherichia coli* O157 by single nucleotide polymorphisms. Genome Res. 16, 757.
Zhu, L.X., Wang, D., Zhang, G.B., Jiang, D., Zhang, Z.W., Zhang, Q., Mitchelson, K., Cheng, J., 2007. Development of a base stacking hybridization-based microarray method for rapid identification of clinical isolates. Diagn. Microbiol. Infect. Dis. 59, 149–156.
Zuker, M., 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31, 3406–3415.