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Oligonucleotide microarrays in microbial diagnostics

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Oligonucleotide microarrays offer a fast, high-throughput alternative for the parallel detection of microbes from virtually any sample. The application potential spreads across most sectors of life sciences, including environmental microbiology and microbial ecology; human, veterinary, food and plant diagnostics; water quality control; industrial microbiology, and so on. The past two years have witnessed a rapid increase of research in this field. Many alternative techniques were developed and validated as seen in 'proof-of-concept' articles. Publications reporting on the application of oligonucleotide microarray technology for microbial diagnostics in microbiology driven projects have just started to appear. Current and future technical and bioinformatics developments will inevitably improve the potential of this technology further.

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Abbreviation
MDM microbial diagnostic microarray

Introduction

Microarrays are a powerful tool for the parallel, high-throughput detection and quantification of many nucleic acid molecules. DNA microarrays (originally developed for the analysis of whole genome gene expression) have considerable potential for applications in microbiology. Depending on the availability of appropriate probe sets, they enable the detection of up to several thousand microbial strains, species, genera or higher clades (depending on the design of the probe) in a single assay.

Microbial diagnostic microarrays (MDMs) (related terms in use are identification arrays, phylochips, phylogenetic oligonucleotide arrays, functional gene arrays and genotyping arrays) fall into two distinct categories according to their intended use. Environmental MDMs [1,2] are applied in environmental and industrial microbiology to obtain a picture of the structure of the microbial community being analysed. Requirements for this class of MDMs are the parallel detection of many microbes at the level of species, genus or even higher taxon and the potential for some level of quantification. Detection/identification MDMs applied in clinical (medical, veterinary, food and biodefense) microbiology [3,4] are, conversely, required to enable the reliable detection and/or identification at the species/subspecies/strain level of one or a few microbes out of many that may be present.

There are many recent technical developments offering alternative solutions for most aspects of MDM technology. While the first publications came from the field of environmental microbiology [5], most of the recent developments originate from clinical microbiology [6,7,8••]. Methods and techniques are, however, easily transferable between these two fields of research. Techniques and 'tricks' have also been 'borrowed' from work on single nucleotide polymorphism analysis [9•].

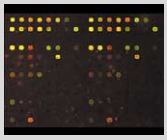
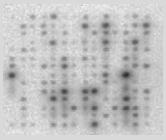
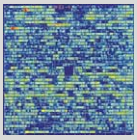
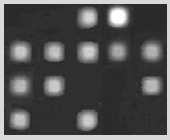
In this review, we summarise recent technological developments in oligonucleotide microarrays for microbial diagnostics with emphasis towards environmental microbiology. We review the field from the point of view of planar glass microarrays because of their widespread use, keeping in mind that the recent rapid developments will probably change many aspects of this type of research in the next few years.

Platforms and technical details

Different platforms currently used for MDMs are summarised in Table 1. Macroarrays (dot blots on nitrocellulose or nylon membranes) have the disadvantage of moderate throughput and uncontrolled binding of oligonucleotides [10,11]. A modification to this technique, reverse line blot hybridisation [12] enables reasonable throughput if the number of probes is limited to about 10–20. With the widespread availability of microarray core technologies, planar glass microarrays have become the most widely used type of array, owing to their general utility and moderate price. Affymetrix microarrays enable very high probe density (well over 100,000 probes per chip) The technology (photolithography) applied determines a high setup cost for any new Affymetrix microarray, even for modified/updated arrays. Their high price and low flexibility, in combination with the lack of a suitably high number of validated oligoprobes currently limits their application in microbial diagnostics. Specific, three-dimensional microarray formats, such as the gel-pad platform [5] or the Pamgene system (www.pamgene.com) offer the option for real-time detection. Coupled with a

Table 1

Platforms in use for microbial diagnostic microarrays.

	Planar glass microarray	Macroarray	Affymetrix gene-chip	Three-dimensional platforms[a]
Density	Low/Medium	Low/Medium	High	Low/Medium
Open access	+	+	−	−
Flexibility	+	+	−	+/−
Price	Moderate	Moderate	High	?
Throughput	High	Moderate	High	High
Real-time detection (on-chip melting curve analysis)	−	−	−	+
				

[a]Gel-pad microarrays (Magi-Chip® or IMAGE Chip); Pamgene®

gradual increase of the hybridisation stringency they enable the on-chip determination of melting curves for each individual oligoprobe on the array, making the design of probe sets significantly easier. These systems are currently available to only a few laboratories.

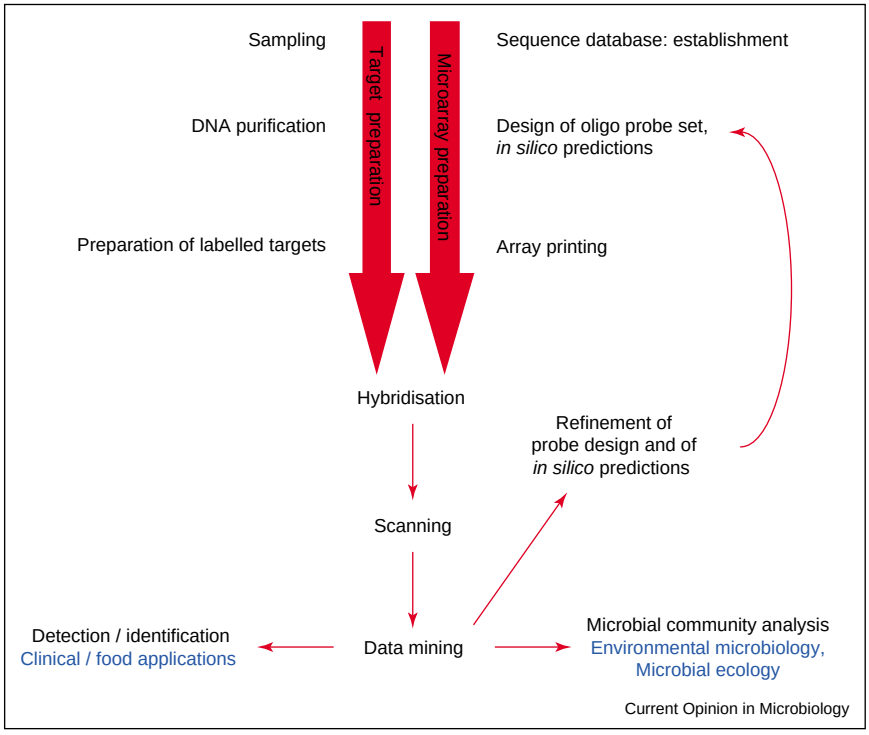
Figure 1 shows a universal scheme for the experimental approach to micro-array work. Oligonucleotides are in most cases immobilised covalently at their 5′ ends to active groups on the coating layer of the glass slide. Spacers are applied to minimise the interference of steric hindrance with hybridisation [13]. Sets of oligonucleotide probes have to be fine tuned for nearly identical melting temperatures. This is either achieved by designing probes of the same length and the addition of tertiary amine salts in the hybridisation buffer [14••] or by manipulating the melting temperature of the oligos (predicted for 'traditional' hybridisation buffers) by changing their lengths [15]. Oligonucleotide probes designed with all possible care often still display two orders of magnitude difference in maximal hybridisation capacity. The directed modification of spacer lengths has recently been successfully applied to decrease this variation [13].

Resolution, specificity and sensitivity

The ultimate resolution of MDMs depends on the level of conservation of the marker gene applied. The 16S rRNA gene is the most widely used marker, however, it does not allow for resolution below the species level. In many cases (e.g. *Enterobacteriaceae*) even species-level differentiation is hardly possible. Alternative universal marker genes with higher resolution include *rpoB*, *recA*, *gyrB*, *groEL*, *atpD* or the tmRNA gene [16]. The application of functional genes as marker genes focuses the array to a defined physiological group of microbes and also broadens the range of investigation to uncultivated members of the same group [17••]. This is achieved because, in contrast to the application of the 16S rRNA, environmental sequences belonging to a novel, but functionally related phylum (i.e. identified by carrying the functional gene in question) can easily be recognised and included in the analysis. For more detailed data and references on applicable marker genes, please refer to www.diagnostic-arrays.com/approach/genes.htm. The need for subspecies-level resolution genetic markers is obvious in clinical applications, but also in environmental microbiology a species-level resolution does not necessarily carry sufficient information. In the nested probe approach, probes of different taxonomic level are combined, increasing the confidence of the assay and enabling the detection of novel members of known groups [18].

The ultimate specificity in MDM technology is discrimination between a perfect match and a single mismatch target. With the oligoprobes that are typically used (20–25 nt in length), this specificity is hard to achieve based solely on hybridisation. Recently, enzyme-assisted detection strategies (also used in single nucleotide polymorphism analysis and resequencing) have gained attention because of their significant discriminating power. While hybridisation discriminates best mismatches in central positions of the probes, enzyme-mediated detections are very sensitive to end-position mismatches. Sequence-specific extension of oligonucleotides with a single, labelled di-deoxynucleotide, followed by hybridisation to complementary oligoprobes has been shown to deliver high specificity and sensitivity [19]. Busti *et al.* [20] combined ligase detection reaction with hybridisation to a universal microarray; tags complementary to the universal probes were included in the oligonucleotides to be ligated. Gharizadeh *et al.* [9•] used sequence specific extension, combined with the application of apyrase (for enhanced selection against 3' mismatch hybridisations) to generate targets that were also hybridised to a universal array. Hybridisation to specific oligoprobes followed by on-chip sequence-specific extension and labelling of the immobilised probes yielded reproduci-

Figure 1



Schematic of the experimental approach. In the validation phase, pure cultures or environmental clones are used to generate targets, and results are compared to expected (*in silico* predicted) hybridisation results.

ble, specific signals [6]. On-chip PCR and on-chip ligase detection reaction have been successfully applied for detecting rifampin-resistant *Mycobacterium tuberculosis* mutants on gel-pad microarrays [21].

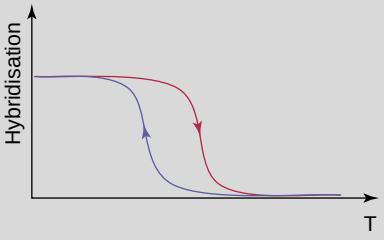
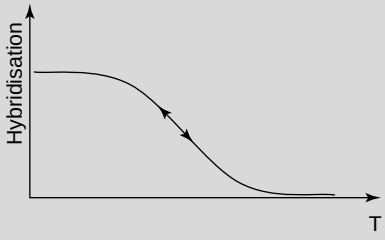
The sensitivity of MDMs is usually defined as the lowest relative abundance of the target group detectable (within the analysed community). This is in the range of 1–5% for 'conventional' planar glass MDMs [17••,22•,23•]. In most cases, the target consists of labelled gene fragments (several hundred nucleotides long), which increases the potential for the accumulation of background signal arising from a low rate of non-specific hybridisation.

PCR amplification of the marker gene(s) is applied to achieve detection with short oligoprobes [7,14••,17••,24•, 25,26•,27]. PCR is required in these cases to focus the labelling to the marker genes (thus decreasing the accumulation of non-specific hybridisation signal as detailed above). Naturally amplified RNA molecules (rRNAs and the tmRNA [28]) offer a potential for PCR-free, direct detection, thus avoiding the inherent bias in consensus PCR [29•,30•,31,32••]. Consensus amplification of target genes is in fact a special case for multiplex PCR. A multiplex quantitative PCR technique [33] was developed for multiplex detection of DNA from genetically modified organisms on microarrays. This approach also shows promise for minimising PCR bias in amplifications with consensus primers. Chizhikov *et al.* [34] used relaxed annealing conditions to achieve multiplex amplification.

Long (50–70mer) oligoprobes display fundamentally different hybridisation properties (Table 2) [35,36]. Microarrays constructed of long oligoprobes are widely used in transcriptomics where detection from a total transcriptome background is required. The sensitivity of these microarrays is several orders of magnitude above that of the 1–5% found for short oligo MDMs. This also holds promise for detection with improved quantification potential via direct labelling of purified environmental DNA, thus avoiding PCR and its inherent bias. A universal viral 70mer microarray was able to detect and correctly classify viruses from human clinical samples [8••]. In this case, multiple genes were used as markers and a universal amplification strategy was applied. The disadvantage of long oligoprobes is their decreased specificity (threshold for differentiation at 75–87% sequence similarity [37,38]). This makes it necessary to target multiple marker genes (specific for the different microbes to be detected/identified), even if universal markers are available. A universal, bias-free amplification and labelling strategy is then required to achieve detection [39–41]. Alternatively, purified environmental DNA can be labelled without amplification. Denef *et al.* [22•] achieved

Table 2

Long versus short oligoprobes.

	Long	Short
Typical length	50–70	20–25
Optimal hybridisation conditions	1 M Na^+, 65–70°C, no formamide	1 M Na^+, 40-60°C, no formamide
Optimal wash conditions	0.015 M Na^+, 65°C	0.015 M Na^+, 20–22°C
Hybridisation kinetics	Irreversible	Reversible
		
Melting temperature is at which...	50% of the nucleotides are unbound (T_m^∞)	50% of the strands are single stranded (T_m)
Dissociation is...	intramolecular process → T_m^∞ is concentration independent	intermolecular process → T_m is concentration dependent

a detection sensitivity of 1% of total community without amplification of the target gene (i.e., within a 100× genomic background), using a 70mer microarray coupled with TSA (tyramide signal amplification). Tiquia *et al.* [23•] achieved a 5% detection limit using 50mer probes and total environmental DNA as target (the same limit was observed by Denef *et al.* [22•] if TSA amplification was avoided). Another strategy to improve detection sensitivity is the application of single mismatch negative controls for each probe (perfect match/mismatch pairs) to filter out most of the non-specific background noise [13]. Chandler *et al.* [30•] applied a two-probe chaperone detection system, consisting of an immobilised, species-specific capture probe and a labelled proximal detector (chaperone) probe for the direct detection of 16S rRNA from environmental samples. The chaperone probe in this system was found to improve specificity and sensitivity by relieving target secondary structure. Peplies *et al.* [13] investigated the applicability of fluorescence *in situ* hybridisation (FISH) probes and helper (chaperone) oligos for MDMs. Helper oligos in their system increased background and were thus omitted.

Data analysis and quantification

The widespread use of MDMs requires a solid bioinformatics background, primarily in two fields. These are reliable prediction of hybridisation [17••,42,43••] and software assisted result analysis [14••]. Visualisation of results with software used in transcriptomics can help to interpret complex results by displaying global trends and patterns [8••,44••].

A simple and elegant method for improved quantification [45] was applied using gene fragments as probes. Each probe was 'spiked' with a common reference gene fragment. Hybridisation was carried out with a Cy3-labelled environmental mixture and a Cy5-labelled reference DNA. Quantification was based on the ratios of Cy3 to Cy5. Unfortunately, the same principle cannot be applied to the quantification of oligonucleotide chip results because of the inherent differences in hybridisation efficiencies between oligonucleotide probes. In practice, a different reference oligo for each probe should be designed. Improved quantification potential was achieved using another two-colour hybridisation strategy, where a Cy5-labelled reference target of known composition was applied in addition to the Cy3-labelled sample target [17••]. Tiquia *et al.* [23•] found a good linear relationship between target DNA and signal on a 50mer microarray.

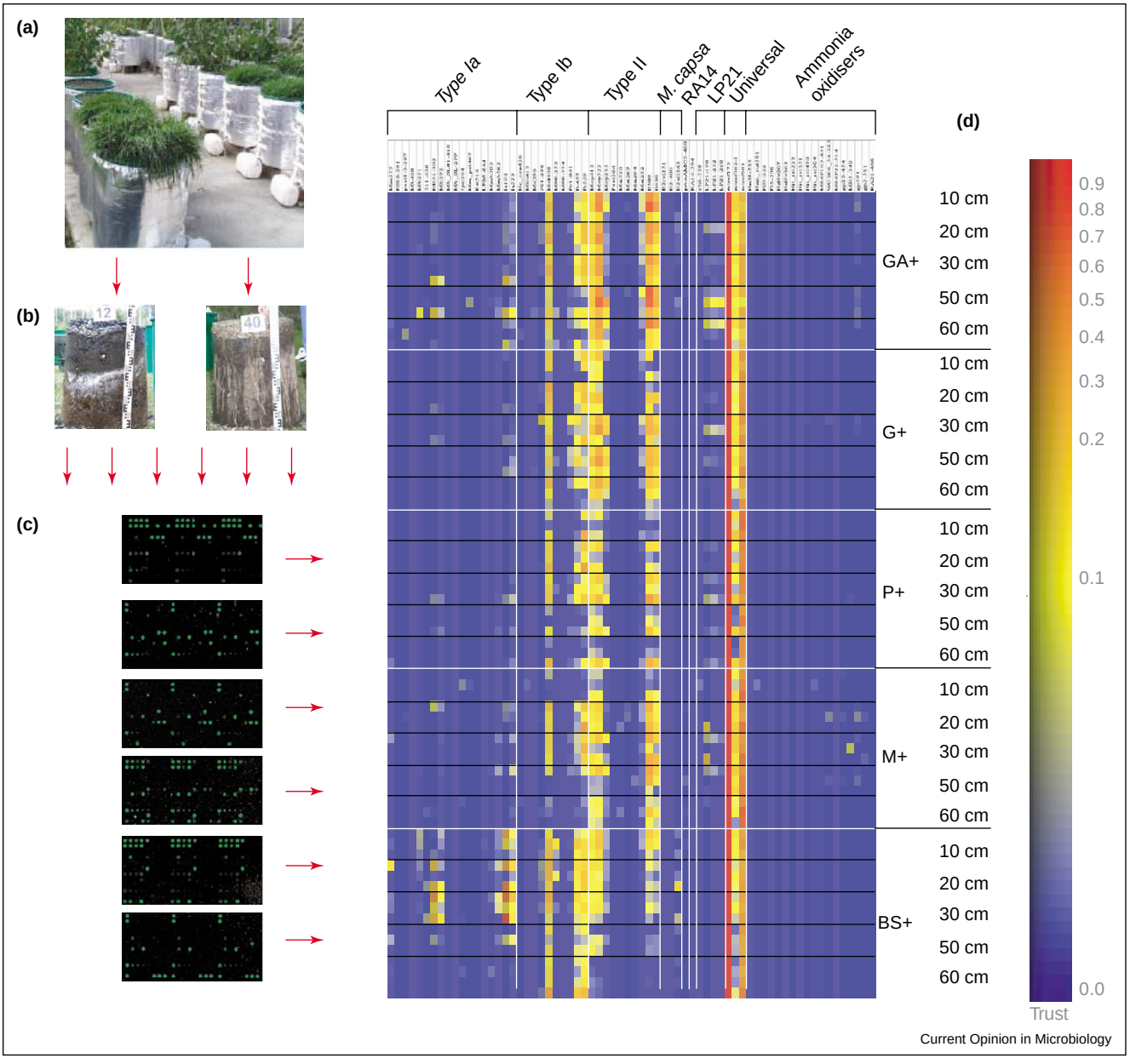
Applications

The application of microarray technology for microbial diagnostics is a field in the stage of dynamic development, with many options available and with advantages and disadvantages associated with each option. The number of published studies is increasing exponentially in this field, but, not too surprisingly, the majority of them are of technical nature while only a few proof-of-concept application experiments have been published.

Oligonucleotide probe sets spotted onto nylon or nitrocellulose membranes ('macroarrays') have been used for the diagnosis of bacteraemia [10], food-contamination [11], detection of enterococci [18] or cyanobacteria [46]. The gel-pad microarray platform was used for the characterization of aromatic hydrocarbon degrading consortia [26•], identification of rifampicin resistant strains of *Mycobacterium tuberculosis* [21] and for the analysis of the thermophilic microbial communities of oil reservoir formation waters [24•]. A chip with a set of 15 probes

targeting the *crm*B genes was developed for the species-specific identification of orthopoxviruses [47]. Different genotypes of rotaviruses and *Listeria* were specifically detected by oligonucleotide microarrays [48] and [49], respectively. *gyrB* based short oligoprobe microarrays were applied to detect and identify closely related enteric bacteria and *Mycobacterium*, respectively [16,50•]. An *hsp*70 single nucleotide polymorphism microarray was used to detect *Cryptosporidium* and to genotype *Cryptosporidium* isolates [51]. 40mer oligoprobes based on the 16S rRNA gene were used to detect 20 common bacterial species of the human intestinal flora [27]. An Affymetrix GeneChip with over 30 000 16S rRNA targeting oligo probes was used to identify culture collection species and subsequently to characterize populations of airborne bacteria at the level of higher phylogenetic taxa [25]. The

Figure 2



Full-scale application of MDM technology. Over one hundred soil samples from landfill-site simulating lysimeters with different plant covers were analysed at a high phylogenetic resolution with respect to their methanotroph communities [44••]. **(a)** Lysimeters used during the experiment. **(b)** Dismantled lysimeters. **(c)** Individual microarray hybridisations. **(d)** Colour-coded table summarising and visualising results. Columns represent oligoprobes and rows represent individual samples. Colour bar indicates relative intensity of the probes (1 = maximum signal obtained during validation with pure reference strains or clones). GA, G, P, M and BS refer to different plant covers (grass-alphalpha, grass, poplar, *Miscanthus* and bare soil, respectively).

same platform was used for the development of a high sensitivity pathogen detection microarray, targeting 19 potential biowarfare agents. For each microbe, 3–10 diagnostic regions were identified and each region was covered by 100–300 20mer oligoprobes [52•]. A 16S rRNA gene based oligonucleotide microarray targeting and covering the entire known diversity of sulphate reducers was developed and successfully validated with environmental samples [14••]. The full potential of MDMs was demonstrated by analysing the methanotroph community of over 100 soil samples with a *pmoA* based microarray (Figure 2) [44••]. Applications of MDMs are summarised in Table 3.

The apparent lack of full-scale application of MDMs in microbiology driven projects is attributable to a rather

Table 3

Applications of MDMs.

Platform	Organisms targeted	Marker gene	Phylogenetic resolution	Oligos	Number of probes	Field[a]	Number of samples analysed (reported)	Environment	References
Macroarray	Bacteraemia causative agents	23S rRNA	Species	20–30	30	C	158	Blood samples	[10]
Macroarray	*Enterococci*	16S and 23S rRNA	Species	18	51	C	1	Wastewater treatment plant	[18]
Macroarray	Cyanobacteria	16S rRNA	Genus	22–31	10	E	8	Lake waters	[46]
Gel-pad	Rifampin resistant strains of *Mycobacterium tuberculosis*	*rpoB*	Strain	15–23	43	C	31	Clinical samples	[21]
Microarray	Human group A rotaviruses	VP7	Genotype	18–26	50	C	NA	NA (isolates genotyped)	[48]
Macroarray	Aromatic hydrocarbon degraders	16S rRNA	Species	16–22	9	E	5	Oil-contaminated marine sediments	[26•]
Gel-pad	Orthopoxviruses	*crmB*	Species	12–16	15	C	NA	NA (isolates genotyped)	[47]
Microarray	Sulfate reducers	16S rRNA	Species	132	18	E/C	2	Hypersaline cyanobacterial lake mat; periodontal tooth pocket	[14••]
Macroarray	Lactic acid bacteria, *Oxalobacter*, *Pseudomonas*, *Enterobacter agglomerans*	16S rRNA	Genus	18–29	7	F	2	Packed vegetable salads	[11]
Microarray	*Cryptosporidium*	*hsp*70	Isolates	15	68	E	NA	NA (isolates genotyped)	[51]
Microarray	*Listeria*	*iap*, *hly*, *inlB*, *plcA*, *plcB*, *clpE*	Species	17–33	132	C	NA	NA (isolates genotyped)	[49]
Microarray	Human intestinal flora[b]	16S rRNA	Species	40	60	C	6	Human intestine	[27]
Microarray	Higher level bacterial clades (Third level according to RDP classification scheme)	16S rRNA	Above genus	20	31 179	E	1	Air filtrate	[25]
Microarray	Major potential biowarfare agents (bacteria, viruses, eukaryotes)[c]	Various	Species	20	53 660	B	2 (air filtrates spiked with pathogens)	Air filtrate	[52•]
Microarray	Viruses[d]	Various	Serotype	70	1600	C	6	Clinical samples	[8••]
Microarray	Methanotrophs	*pmoA* (functional)	Species/ subspecies	17–27	61	E	>100	Landfill cover soil	[17••,44••]
Gel-pad	Thermophilic anaerobic *Archea* and *Bacteria*	16S rRNA	Genus	17–20	17	E	3	Oil reservoir formation waters	[24•]
Microarray	*Mycobacterium spp.*	*gyrB*	Species	13–15	28	C	40	Human sputum	[50•]
Microarray	*Escherichia coli*, *Shigella*, *Salmonella*	*gyrB*	Species	15–19	10	C	–	–	[16]
Microarray	Influenza viruses	Various	Subtype	17–29	476	C	NA	NA (isolates genotyped)	[65]
Microarray	*Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*	*fur*, *glyA*, *cdtABC*, *ceuB-C*, *fliY*	Species	17–35	74	C	16+6	Isolates and mixed cultures	[7]
Microarray	Higher taxa (subclusters of α–Proteobacteria and γ–Proteobacteria; CFB)	16S rRNA	Higher taxa	15–20	21	E	1	Marine bacterioplankton	[66]

[a]B-biodefense, C-clinical, E-environmental, F-food. [b]No data on validation of the probes. [c]3–10 diagnostic regions were identified per microbe and each one of them was covered by 100–300 probes. [d]70mer discriminatory oligoprobes were designed based on all published viral genome sequences.

slow transition of the field from the stage of technology development to that of application. This is partly due to the highly complex problem of optimising the method considering all the different options available and the advantages/disadvantages associated with them. The other reason is, however, that MDMs allow microbiologists (especially in microbial ecology) to ask questions which could not be asked before. Now one can obtain information within a month, which would have taken three years of a PhD student a few years ago. To get the maximum out of the potential of MDMs, one needs to rethink the way questions are asked and experiments are designed. Finally, new approaches are needed to extract the meaningful information from the vast amount of data that can be generated with MDMs.

Related technological developments

The technological platforms currently used for MDMs are excellent research and development tools, opening new horizons in microbiology. A number of alternative technologies are, however, emerging which may well supersede the current ones in the future. Various platforms based on microbeads (suspension arrays, fiber bead arrays and capillary bead arrays) have the common advantage of higher throughput, easy agitation during hybridisation and decreased steric hindrance [53–55]. Electronic [56–58] and cantilever microarrays [59] offer a cheaper, label-free detection platform.

Novel imaging technologies may also improve optical detection. Evanescent field based detection enables time-resolved detection of hybridisation events, allowing for extremely fast association-phase detection or high accuracy dissociation-phase detection (reading each probe at its individual melting temperature) [60]. Surface plasmon resonance imaging allows for label-free detection and also enables the re-use of the array [31].

Locked nucleic acid is an artificial nucleic acid analog with increased binding strength. Locked nucleic acid monomers can be incorporated into DNA oligomers enabling the manipulation of the hybridisation behaviour of the resulting probes [61].

One of the key issues in microbial ecology is linking function to phylogeny. In the isotope array approach, environmental samples are 'fed' with a radioactively labelled compound, which gets incorporated into the DNA or RNA of the microbial population using it. Phylogenetic identification is then coupled with radioactive detection, thus linking function to phylogeny [32••,62]. Stable isotope probing [63] allows for the separation of the DNA of a group of microorganisms utilising a given substrate from the bulk of the environmental DNA. Combining this approach and MDMs holds promise in identifying the environmental function of the microbes detected with a phylogenetic marker.

Conclusions

As seen above, technologies used for MDMs are rather diverse. In addition to planar glass microarrays, filter macroarrays, special 3-dimensional platforms and Affymetrix gel chips are applied. The 16S rRNA is still the most widely used marker gene, but a number of higher resolution and/or non-universal (i.e. focused on a narrower group of microbes) genes are also being used as markers. Short oligoprobes, applied in a hierarchical and parallel manner, are the preferred choice in microbial ecology. Long oligoprobes, more frequently used in clinical microbiology, are also now being used in environmental microbiology. The latter approach targets multiple marker genes, coupled with universal amplification techniques. The labelled target is either DNA or RNA; some laboratories fragment their targets while others do not. The generation of the target nucleic acid is usually achieved by PCR. Amplification-free detection is currently applied only to rRNA. Hybridisation is carried out in diverse solutions, including those minimising the effect of GC content on hybridisation. Overnight incubation with some kind of agitation is usually performed to obtain complete, reproducible hybridisation. New hybridisation equipment enables a strict control over hybridisation temperature throughout the entire procedure. Hybridisation is carried out in small volumes of 25–50 µl when static; agitating usually requires a compromise in volume, thus diluting out labelled target. This is however compensated for, both in uniformity and in signal strength, by agitation. Cy or Alexa form the basis of most dyes for labelling. Dyes are incorporated either directly or via aminoallyl dNTPs during PCR or *in vitro* transcription. A variety of alternative labelling techniques have been proposed to improve various limitations of the more 'traditional' approach. These include sandwich-type hybridisations with capture and detector oligos and enzyme-mediated labelling strategies for improving specificity [9•,19,30•].

Depending on the marker genes applied, current MDMs can provide resolution at various taxonomic levels, depending on the requirements. MDMs with oligoprobes from a few tens to tens of thousands have already been developed. Reliable community analysis of 10–30 samples can be achieved within 24 hours by one researcher. For clinical application, where complete hybridisation is not an important issue, detection is possible within a working day. Results can be quantified on a comparative basis if similar samples are analysed. Absolute quantification is troubled by biases inherent in PCR, in DNA recovery and in different numbers of gene copies per genome. The sensitivity of MDMs is normally limited by the relative abundance of the microbial population within the targeted community, with reported detection limits being 1–5%. The hybridisation potential of the oligoprobes is predicted based on the nearest neighbour model and a number of empirical microarray-specific factors.

The perspective of fast, high-throughput detection and identification of hundreds to thousands of microbes via MDMs promises significant advancements in most fields of microbiology. Current limitations and options to solve them are detailed below.

A further improvement in the prediction of the hybridisation behaviour of the oligoprobes requires more input from bioinformatics and the establishment of datasets to understand array-specific effects such as immobilisation, steric hindrance, and so on. In many cases, especially for clinical diagnostics, a very high resolution of detection/identification is needed. This requires the application of less conserved markers. Large sequence databases of such markers (comparable in size to the 16S rRNA database) are badly needed. Linking this sequence information to phylogenetic traits (i.e. pathogenicity and antibiotic resistance in clinical microbiology; environmental parameters, temperature and growth substrates in environmental microbiology) will enable the prediction of these functions, at least at a given level of certainty, from microarray results. There are applications where PCR is not acceptable, either because the bias introduced is too high or simply not acceptable, or because only live bacteria need to be detected. The direct detection of naturally amplified RNA species (rRNAs, tmRNA) is a logical solution here. Two-colour hybridisations with an artificial mixture of known composition as reference target can significantly improve the quantification potential of MDMs (while not curing the above mentioned inherent biases). Enzyme mediated alternative labelling techniques, improving the specificity of the approach, should enable higher detection sensitivity, perhaps as low as 0.1% of the total microbial community. Artificial nucleic acid homologues with enhanced binding capacities also hold promise here. In clinical and food diagnostics, timescale is often a crucial factor. The most time-consuming steps in the current technologies are PCR amplification and hybridisation. Detection of naturally amplified RNA species and the application of online hybridisation detection, enabling association-phase detection, may decrease this time from about eight hours to less than two hours. Oligoprobe design is often limited by the length, GC content, number and position of diagnostic residues within a diagnostic region. The incorporation of artificial DNA/RNA analogues with modified hybridisation properties in key positions of oligoprobes will relax some of these limitations. The combination of radioactive or heavy (non-radioactive) isotopes with MDMs will help to understand the physiological role of the microbes detected and identified at the phylogenetic level.

Update

A microarray with 32 oligoprobes targeting the 23S rRNA gene was developed and successfully applied to detect veterinary pathogens responsible for equine abortion from clinical samples. On-chip PCR was applied to achieve a discrimination against single mismatch targets [64].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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