New Phytologist

Review

Tansley review

Diagnostic microbial microarrays in soil ecology

Author for correspondence:
Angela Sessitsch
Tel: +43 50550 3509
Fax: +43 50550 3666
Email: angela.sessitsch@arcs.ac.at

Received: *2 March 2006*
Accepted: *12 May 2006*

A. Sessitsch[1], E. Hackl[1], P. Wenzl[2], A. Kilian[2], T. Kostic[1], N. Stralis-Pavese[1], B. Tankouo Sandjong[1] and L. Bodrossy[1]

[1]ARC Seibersdorf research GmbH, Department. of Bioresources, A–2444 Seibersdorf, Austria;

[2]DArT P/L, PO Box 7141, Yarralumla, ACT 2600, Australia

Contents

Key words: community genome array (CGA), functional gene array (FGA), functional markers, high throughout population analysis, microbial diagnostic microarray (MDM), phylochip, phylogenetic markers.

Summary

Soil microbial communities are responsible for important physiological and metabolic processes. In the last decade soil microorganisms have been frequently analysed by cultivation-independent techniques because only a minority of the natural microbial communities are accessible by cultivation. Cultivation-independent community analyses have revolutionized our understanding of soil microbial diversity and population dynamics. Nevertheless, many methods are still laborious and time-consuming, and high-throughput methods have to be applied in order to understand population shifts at a finer level and to be better able to link microbial diversity with ecosystems functioning. Microbial diagnostic microarrays (MDMs) represent a powerful tool for the parallel, high-throughput identification of many microorganisms. Three categories of MDMs have been defined based on the nature of the probe and target molecules used: phylogenetic oligonucleotide microarrays with short oligonucleotides against a phylogenetic marker gene; functional gene arrays containing probes targeting genes encoding specific functions; and community genome arrays employing whole genomes as probes. In this review, important methodological developments relevant to the application of the different types of diagnostic microarrays in soil ecology will be addressed and new approaches, needs and future directions will be identified, which might lead to a better insight into the functional activities of soil microbial communities.

New Phytologist (2006) **171**: 719–736

doi: 10.1111/j.1469-8137.2006.01824.x

I. Introduction

Soil is an environment that is characterized by high heterogeneity and extremely diverse microbial populations, which are responsible for important physiological and metabolic processes (Alexander, 1977). The genetic diversity of several soils was studied by reassociation kinetics (Torsvik *et al.*, 1990, 1996; Øvreås & Torsvik, 1998), leading to the identification of up to 10.000 genome equivalents in undisturbed soils and 350–1.500 genome equivalents in arable or heavy metal polluted soils (Torsvik & Øvreås, 2002). The phylogenetic diversity of soil microorganisms and particularly their functional potential, however, are to a large extent unknown. This is because only a minority of microorganisms can be cultivated (Amann *et al.*, 1995), and the fraction of readily culturable cells tends to be unrepresentative of the total microbial community (Dunbar *et al.*, 1999; Kaiser *et al.*, 2001; Smit *et al.*, 2001). Most soil microbes do not grow on standard cultivation media and novel strategies are being developed in order to cultivate and further analyse previously uncultured microbes (Joseph *et al.*, 2003; Svenning *et al.*, 2003; Stevenson *et al.*, 2004; Sangvan *et al.*, 2005).

Cultivation-independent molecular analysis of soil microbial communities has been widely performed because issues of microbial diversity, community patterns and community shifts can be addressed without the bias that is associated with cultivation-based analyses. Generally, novel microorganisms are detected and included in the analysis so that time-consuming steps involved in the cultivation of microbes are avoided. Molecular methods are frequently based on the use of ribosomal genes as phylogenetic markers, with the advantage that a large number of sequences are available in public databases. Nucleic acids can be directly isolated from terrestrial environments and are used as templates in polymerase chain reaction (PCR)-mediated amplification of phylogenetic marker genes. Sequence analysis is used in order to obtain taxonomic information on soil microorganisms, whereas community 'fingerprinting' tools such as denaturing gradient gel electrophoresis (DGGE) or terminal restriction fragment length polymorphism (T-RFLP) analysis are now routinely applied to follow population changes under particular conditions. Such studies have identified the major bacterial phylogenetic groups in numerous soils, namely Alphaproteobacteria, high G + C Gram-positive bacteria, *Acidobacteria* and *Verrucomicrobia*, and have provided insight into the relations of soil microbial communities with soil type, agricultural practices, vegetation composition and climatic factors (Kuske *et al.*, 2002; Hackl *et al.*, 2004; Lipson *et al.*, 2005). The taxonomic variability of microbial communities has been linked with the analysis of functional genes and activity measurements in order to obtain an understanding of the functional roles of natural microbial communities. Molecular tools based on 16S rRNA or functional genes have also taken the heterogeneity of soils into account, revealing extremely high microscale diversity (Grundmann & Normand, 2000; Sessitsch *et al.*, 2001).

Cultivation-independent community analyses have revolutionized our understanding of soil microbial diversity and population dynamics. Nevertheless, many methods are still laborious and time-consuming, and frequently detailed analysis and characterization of microbial populations responding to different treatments or within replicates is not feasible using present-day standard techniques for molecular community analysis. Owing to the high versatility and rapid adaptation of microbial populations and the high heterogeneity and microscale diversity in soils, high-throughput methods have to be applied in order to understand population shifts at a finer level and to be better able to link microbial diversity with functioning of ecosystems. Microarrays represent a powerful tool for the parallel, high-throughput identification of many microorganisms. They were originally developed for the analysis of gene expression in a variety of model organisms, but have great potential in microbial diagnostics, community analysis and in the detection of different functional characteristics and their expression.

Microbial diagnostic microarrays (MDMs) (related terms are identification arrays, phylochips, phylogenetic nucleotide arrays, functional gene arrays and genotyping arrays) may comprise up to several thousand probes, which are, depending on the design of the probes, specific for different strains, species, genera or higher taxa (Bodrossy & Sessitsch, 2004; Loy & Bodrossy, 2006). Microbial diagnostic microarrays therefore allow the parallel detection of many microorganisms and find application in many areas such as clinical, veterinary, food and biodefence microbiology (Stenger *et al.*, 2002; Clewley, 2004). In these applications usually few, well-defined microorganisms have to be reliably detected and identified, whereas in environmental studies generally the whole community structure is of interest. Microarrays have proven useful to study soil microbial ecology, however, because of the high complexity of the soil microflora and the high biomass, the frequently high concentration of substances inhibiting enzymatic reactions, and the novelty of the technology, many studies are of technical nature. Optimal conditions are being elaborated in order to improve sensitivity and selectivity of the analysis, and suitable probes are developed in order to detect either specific phylogenetic groups or relevant functional genes.

In this review, we address important methodological developments relevant to the application of diagnostic microarrays in soil ecology. The application of MDMs to obtain better information on the diversity and functionality of soils will be reviewed and microarray-based results giving new insights in soil ecology will be highlighted. Finally, new approaches, needs and future directions will be identified, which may lead to a better insight into the functional activities of soil microbial communities.

II. General methodology

The first and still most used format for microarrays are planar 1×3 inch (approx. 2.5×7.6 cm) glass slides. Oligonucleotides are in most cases bound via their 5′ ends to reactive groups on the coating layer of the glass surface. Conversely, gene fragments are usually immobilized in a less described, more or less random manner. The establishment of microarray core facilities (including microarray spotting and detection devices) in many laboratories and the general utility, flexibility and moderate price are mainly responsible for the success of this standard format. Other formats, offering clear advantages, such as much higher probe density (Wilson *et al.*, 2002) or a time-course analysis of the hybridization event (Urakawa *et al.*, 2003; Anthony *et al.*, 2005) are currently available only in a few laboratories.

Three main categories of MDMs for microbial community analysis have been defined (Zhou, 2003) based on the nature of probe and target molecules used (Table 1):

- Phylogenetic oligonucleotide microarrays (phylochips), with short oligonucleotides designed against a phylogenetic marker gene (such as the 16S rRNA gene or *gyrB*).
- Functional gene arrays (FGAs), using gene fragments or oligonucleotides targeting genes of the function of interest as probes.
- Community genome arrays (CGAs), employing whole bacterial genomes as probes.

1. Phylochips

Phylochips contain short oligonucleotide probes, targeting phylogenetic marker genes (evolutionary conserved genes, the sequence of which reflect the phylogenetic relationship of the carrying organisms). The most widely employed target molecule for the detection and phylogenetic analysis of microorganisms is the small-subunit ribosomal RNA (SSU rRNA) and its gene (Guschin *et al.*, 1997; Ludwig & Schleifer, 1999; Loy *et al.*, 2002; Wilson *et al.*, 2002; Loy *et al.*, 2003; Warsen *et al.*, 2004; Sanguin *et al.*, 2005). This is also reflected in the existence of large and regularly updated sequence (Cole *et al.*, 2003; Ludwig *et al.*, 2004) and probe databases (http://www.microbial-ecology.net/probebase/) (Loy *et al.*, 2003) for this target molecule. The main limitation of using the SSU rRNA (gene) as a marker in microarray assays is that its phylogenetic resolution is limited at, or, in some cases, above the species level. Alternative probe targets with a species-level resolution include the large-subunit ribosomal RNA (LSU rRNA) (Anthony *et al.*, 2000; Mitterer *et al.*, 2004; Lehner *et al.*, 2005), the SSU–LSU rRNA intergenic spacer region (Cook *et al.*, 2004; Nübel *et al.*, 2004; Günther *et al.*, 2005) and various house-keeping genes such as *rpoB* (Mollet *et al.*, 1997; Dahllöf *et al.*, 2000; Drancourt *et al.*, 2004), *gyrA* (Brisse & Verhoef, 2001), *gyrB* (Kakinuma *et al.*, 2003), *recA* (Lloyd & Sharp, 1993), *tuf* (Ludwig *et al.*, 1993; Baldauf *et al.*, 1996), *groEL* (Wong &

Table 1 Major differences of various types of microarrays for environmental studies

	Long oligonucleotide-based FGAs	PCR-product-based FGAs	Community genome arrays	Phylogenetic oligonucleotide arrays	Short oligonucleotide-based FGAs
Typical probe size	50–100 bp	200–1000 bp	Entire genome, but fragmented	18–28 bp	18–28 bp
Nature of targeted genes	Functional	Functional	Entire genome	Phylogenetic	Functional[1]
Information on functional activity	Yes	Yes	No	No	Yes
Targeted microorganisms	Culturable and nonculturable	Culturable and nonculturable	Culturable, potentially also nonculturable[2]	Culturable and nonculturable	Culturable and nonculturable
Number of different genes targeted	Many	Many	NA	1 (a few, maximum)	1 (a few, maximum)
Taxonomic resolution	Genus–species[3]	Genus-species[3]	Genus–species[4]	Species-strains	Species–strains
Potential to discover novel bacteria	No	No	No	Yes[5]	Yes[5]
PCR amplification of targeted genes	Not required	Not required	Not required	Required	Required
Construction of comprehensive arrays	*In silico* probe design	PCR amplification	Whole genome preparation	*In silico* probe design	*In silico* probe design

FGA, functional gene array; PCR, polymerase chain reaction; NA, not available.
[1]Reflecting phylogeny of a functionally defined microbial community.
[2]Nonculturable if based, for example, on metagenomic libraries.
[3]Strain-level specificity possible if the genes targeted are themselves strain specific.
[4]Recent evidence suggest that subtractive suppression hybridization between strains of the same species may be used to generate strain specific CGAs (Bae *et al.*, 2005).
[5]Based on hierarchical probes sets.

Chow, 2002), *atpD* (Ludwig *et al.*, 1993), *ompA*, *gapA*, *pgi* (Wertz *et al.*, 2003) and tmRNA (Zwieb *et al.*, 2003). However, individual sequence databases for these alternative markers, if they exist at all, currently contain considerably fewer entries than the SSU rRNA databases, constraining the development and evaluation of encompassing probe sets for microarrays (Hashsham *et al.*, 2004).

The fungal internal transcribed spacer (ITS) region could be amplified by using universal fungal primers directed toward conserved regions of the 18S and 28S rRNA and is widely used for the molecular detection and identification of fungi (Druzhinina *et al.*, 2005). The fungal ITS region has been targeted to develop a DNA microarray for detection and clear differentiation of species belonging to *Candida* and *Aspergillus* (Leinberger *et al.* 2005) and for the identification and differentiation of trichothecene producing and nonproducing *Fusarium* species occurring on cereal grain (Nicolaisen *et al.*, 2005).

2. Functional gene arrays

Functional gene arrays (FGAs) contain DNA probes targeting genes conferring a specific function to the carrying microorganisms, such as nitrogen metabolism (Wu *et al.*, 2001; Taroncher-Oldenburg *et al.*, 2003) or methane oxidation (Bodrossy *et al.*, 2003) (for a general procedure see Fig. 1). The probes applied may be short (typically 15- to 30-mer) (Bodrossy *et al.*, 2003; Stralis-Pavese *et al.*, 2004) and long (typically 40- to 70-mer) (Denef *et al.*, 2003; Taroncher-Oldenburg *et al.*, 2003; Tiquia *et al.*, 2004) oligonucleotides as well as PCR-amplified gene fragments (Wu *et al.*, 2001; Cho & Tiedje, 2002; Dennis *et al.*, 2003).

Microarrays (both FGAs and phylochips) based on short oligonucleotides are bound to be applied in combination with a consensus PCR amplifying the target gene from a wide range of (optimally all) microbes (Loy *et al.*, 2002; Wilson *et al.*, 2002; Bodrossy *et al.*, 2003; Bonch-Osmolovskaya *et al.*, 2003). The PCR amplification is required to narrow down the complexity of the labelled target, allowing for the development of detectable signal.

Short oligonucleotide FGAs enable a phylogenetic investigation of a functionally defined microbial community. The phylogeny in this case is that of the functional gene, the validity of which needs to be investigated in advance (i.e. compared with that based on 16S or other established phylogenetic markers). The advantage of this type of MDM is that it focuses the labelling onto this defined group of bacteria, enabling the detection of otherwise minor microbial groups. Furthermore, the presence and existence of as yet unknown members of a functional group may also be indicated (as opposed to rRNA based microarrays) (Fig. 2).

Long oligonucleotide and gene fragment FGAs enable a more limited resolution, thus signals arising from bacteria carrying closely related enzymes are not readily differentiated (Li *et al.*, 2005). By contrast, a range of genes, encoding for enzymes with related functions, can easily be targeted on a single array, which in turn can deliver information on the metabolic potential of the entire microbial community.

3. Community genome arrays

Reverse sample genome probing (RSGP) technique, introduced in the early 1990s, uses entire bacterial genomes (suitably fragmented) as probes on traditional nitrocellulose or nylon membranes and radioactively labelled environmental DNA as target (Voordouw *et al.*, 1991; Greene & Voordouw, 2003). Community genome arrays or CGAs are based on the same principle, using microarrays and fluorescent labelling (Murray *et al.*, 2001; Zhou, 2003). Being based on whole-genome hybridization the method is directly linked to the microbial species concept (> 70% genome homology). By careful adjustment of hybridization conditions, species-level differentiation was achieved (Wu *et al.*, 2004). Subspecies specificity was achieved for *Salmonella* when probes were generated from whole genomes via subtractive suppression hybridization (Bae *et al.*, 2005). However, cross-hybridization between various genomes is complicated and depends largely on the experimental conditions, the genomes used as probes and on the complexity of the environmental sample, and is thus not a trivial task to predict and account for. A major drawback of the CGA approach is that only cultivated bacteria can be used to generate probes. Novel metagenomic techniques generating large genome fragments from uncultivated microbes (Handelsman, 2004) may help in overcoming this limitation, even though the identification of the bacteria harbouring the arrayed genome fragments may still be a challenging task. A clear advantage of CGAs is that there is no need for PCR amplification of the target, avoiding various problems associated with PCR bias. Furthermore, a linear relationship was found between target concentration and signal, offering a good quantification potential (Wu *et al.*, 2004).

III. Methodological considerations

1. Probe set design

The design of a suitable probe set is the first step in microarray development. It is indeed a rather challenging stage, crucial for the success of the project. All probes on a microarray should (i) be highly specific for their target genes, i.e. not cross-hybridize with nontarget sequences (specificity); (ii) bind efficiently to target sequences to allow the detection of low abundance targets in complex mixtures (sensitivity); and (iii) display similar hybridization behaviour (i.e. similar thermodynamic characteristics under the same experimental conditions – homogeneity, uniformity). Unfortunately, these represent conflicting goals in practice, and thus efforts to fulfil these criteria need to be carefully balanced during probe design and experimental procedures. Again, there is a marked

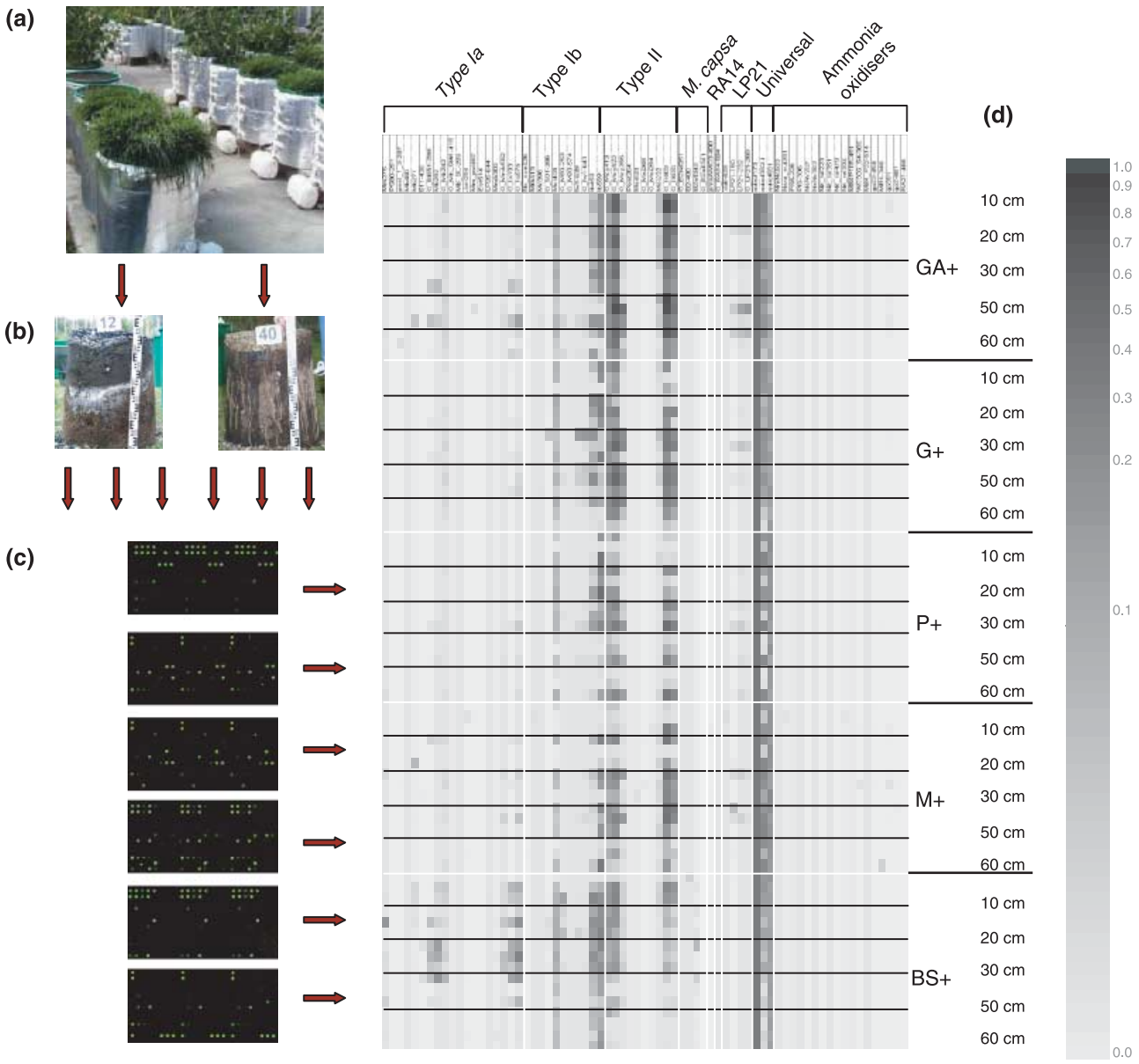

Fig. 1 Analysis of methanotroph communities in soils of a lysimeter experiment simulating landfill conditions. (a) Lysimeter experiment testing the effect of different vegetation regimes on methane oxidation. (b) Sampling soils at different depths of lysimeter pots. (c) Microarray analysis by using a *pmoA* oligonucleotide microarray (Stralis-Pavese *et al*., 2004). (d) Summarized results of the microarray analysis. Results of individual microarray experiments were first normalized to positive control probe mtrof173, then to the reference values determined individually for each probe, averaged between parallels and displayed using the GENESPRING software. In essence, a value of 1.0 indicates maximum achievable signal for an individual probe, while a value of 0.1 indicates that *c*. 10% of the total polymerase chain reaction (PCR) product hybridized to that probe. Grey shading is indicated on the side bar. GA+, Grass–alfalfa mixture; G+, grass; P+, poplar; M+, *Miscanthus*; BS+, bulk soil. For each vegetation cover and each depth layer, results from three parallel lysimeters are shown.

difference between microarrays based on short oligonucleotide probes vs those employing long oligonucleotides or gene fragments. In the following text we summarize the major challenges for short oligonucleotide microarrays, then highlight unique considerations for the development of long oligonucleotide/gene fragment microarrays.

Short oligoprobes have to be fine-tuned for uniform thermodynamic behaviour. One way to achieve this is by using probes that are identical in length and adding tertiary amine salts such as tetramethylammonium chloride to the hybridization and/or washing buffers (Loy *et al*., 2002, 2005). Thereby, differences in the G + C contents and thus duplex stabilities among the probes are attenuated (Jacobs *et al*., 1988; Maskos & Southern, 1992). Another strategy to equalize melting properties of different oligonucleotide probes is to manipulate their length (Bodrossy *et al*., 2003; Stralis-Pavese *et al*., 2004).

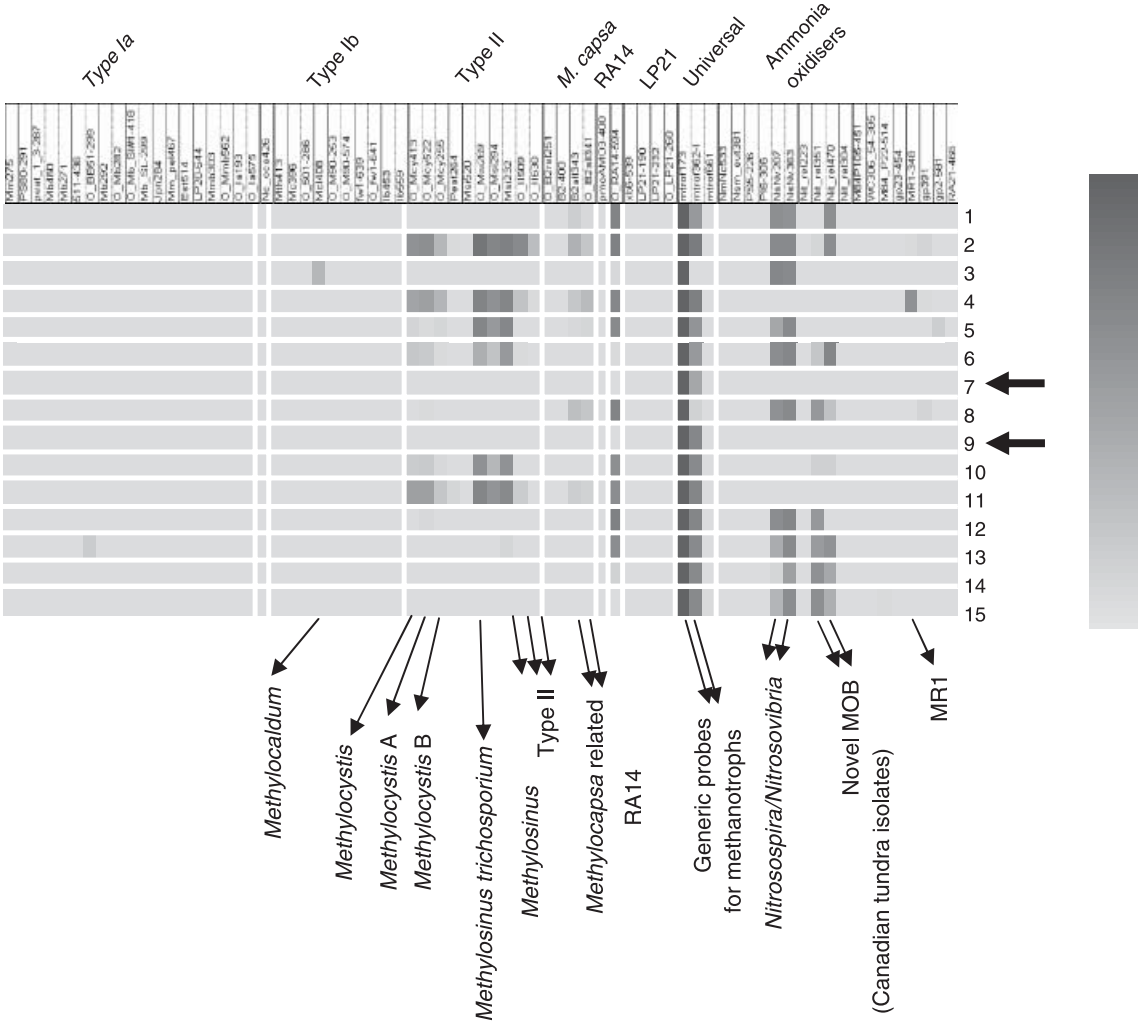

Fig. 2 Analysis of methanotroph communities inhabiting different upland soils by using the *pmoA* microarray. Samples #7 and #9 indicate the presence of novel methanotrophs as hybridization signals were only obtained with generic probes for methanotrophs. Confirmatory results were obtained by analysis of *pmoA* clone libraries (Knief *et al*., 2005).

For single oligonucleotide probes, it is in most cases possible to adjust experimental conditions in such a way that no cross-hybridization occurs. However, the most widely used microarray hybridization formats only allow hybridization and/or washing at a single stringency, making it impossible to provide optimal hybridization conditions for all probes on a microarray. Thus, probes frequently bind to nontarget sequences (false-positive signals) (Loy *et al.*, 2002; Bodrossy *et al.*, 2003). Nonetheless, several approaches still guarantee the reliability of identification by microarrays. One approach involves the design of multiple probes with identical specificities for the same target sequence or group of sequences. Therefore, all probes in a set of probes perfectly matching the target organism must show positive hybridization signals to minimize the risk of false-positive identification (Al Khaldi *et al.*, 2004; Sergeev *et al.*, 2004; Volokhov *et al.*, 2004). This multiple probe concept can be further extended by probes with hierarchical (nested) specificities for the target sequences, also enabling the detection of novel members of known groups (Behr *et al.*, 2000). Probe sets based on rRNA make the application of nested probes easier (Behr *et al.*, 2000; Liu *et al.*, 2001; Loy *et al.*, 2002, 2005), compared with those based on protein-coding genes. This is because the highly variable third codon ('wobble') position in these genes may hamper the design of probes with broader specificity, spanning more distantly related sequences. In addition to, or instead of, the use of multiple probes for one marker gene, it is possible to use more than one marker gene of a target organism as a probe target during hybridization. This multiple probe–multiple target strategy also increases redundancy and hence the confidence in a positive result (Al Khaldi *et al.*, 2004; Volokhov *et al.*, 2004). Furthermore, careful selection

of multiple marker genes may also allow genotyping of the microbes detected. Another way to ensure specificity is the inclusion of so-called mismatch-control probes on the microarray (Wilson *et al.*, 2002; Huang *et al.*, 2004; Lee *et al.*, 2004). Comparison of signal intensities from perfectly matched and mismatched probes allows cross-hybridization to be identified and its extent estimated.

Long oligonucleotides and gene fragments have a higher binding capacity, thus sensitivity is less a problem for microarrays based on them. It is easier to achieve relatively uniform hybridization behaviour for such probe sets as random differences in their GC content are usually balanced across the length of the probes. However, when probes are designed against microbes with markedly different GC content, adjustment of the probe lengths may be necessary. Because single nucleotide differentiation cannot be achieved, specificity has to be based on the overall variability of the gene region targeted. Alternatively, probes may target a broad range of diversity of the targeted gene.

The hybridization properties of short and long oligonucleotide probes are fundamentally different, with consequences for their application potential. While in principle short oligonucleotides allow the discrimination of single nucleotide differences under optimal conditions, this does not hold true for long oligonucleotides. Their threshold for differentiation is approximately 75–87% sequence similarity (Kane *et al.*, 2000; Taroncher-Oldenburg *et al.*, 2003; Tiquia *et al.*, 2004). Long oligonucleotide probes show pronounced hysteresis (higher temperatures for dissociation than for association) (Wetmur, 1991; Hughes *et al.*, 2001). As a direct consequence they display orders of magnitude higher target binding capacities and hence the use of long oligonucleotides should generally improve the detection sensitivity of a microarray (Letowski *et al.*, 2004). Typically, long oligonucleotide microarrays are used in combination with universal (not PCR based, low bias) amplification strategies or without any amplification (Wang *et al.*, 2002; Tiquia *et al.*, 2004; Vora *et al.*, 2004). Probes are then targeting various, nonconserved genes which are specific to the microorganisms to be detected. Thus, the low differentiation power of long oligonucleotide probes is compensated for by the host specificity of the genes they are designed against. With or without universal amplification, the resulting target represents the entire gene pool present in the investigated sample, without any reduction in its complexity. Higher binding capacities of the long oligoprobes and higher target complexity results in similar relative detection thresholds to that of short oligoprobes in conjunction with PCR amplification (approximately 5% of the total microbial community) (Bodrossy *et al.*, 2003; Denef *et al.*, 2003). Improving the detection limit to 1% was recently achieved for long oligoprobe microarrays upon method optimization (Tiquia *et al.*, 2004; Kostic *et al.*, 2005). The FGAs based on PCR-amplified gene fragments display very similar hybridization behaviour to those based on long oligoprobes.

2. Validation

In silico predictions of the hybridization behaviour of microarray probes are limited in their accuracy. This is especially true for short oligoprobes (typically 15–30 nt long). At best, such predictions will deliver a prefiltered set of candidate probes, the specificity of which has to be extensively tested under the intended experimental conditions. In practice, a suitable set of test targets should contain at least one, but ideally three perfectly matched target for each probe on the microarray. After testing the probe set by individual hybridizations with each test target, 'bad' probes showing low sensitivity and/or specificity are removed or replaced. Subsequently, concentration series of targets perfectly matching those probes that have displayed the highest and lowest duplex yield should be hybridized to the microarray to give an impression of the range of sensitivities achievable for the individual probes. (Loy *et al.*, 2003; Stralis-Pavese *et al.*, 2004). Thorough, rigorous validation, involving an evaluation and refinement of *in silico* predictions, as well as the adjustment of hybridization conditions is the key to the development of reliable microbial diagnostic microarrays. Validation, as outlined earlier, may, however, be unfeasible for various reasons. Sequences released in GenBank are frequently not readily available as clones, genomic DNA or microbial isolate. Thorough validation of arrays covering a broad phylogenetic/functional diversity of microbes presents a serious challenge (Franke-Whittle *et al.*, 2005; Sanguin *et al.*, 2005).

The validation of high-density MDMs, consisting of thousands of probes, as described earlier, is clearly unrealistic. Their high probe density, however, offers ample compensation for this. (DeSantis *et al.*, 2005) designed an Affymetrix GeneChip consisting of over 60 000 16S rDNA-based oligoprobes. The array contained mismatch control probes for each (perfect match) probe and a very high probe redundancy, resulting in a robust, sensitive and high-resolution characterization of the composition of airborne bacteria.

The hybridization specificity of long oligoprobes and gene fragments is more predictable (He *et al.*, 2005). Such microarrays, if the probes are targeting genes with little sequence homology (i.e. less, then 70% sequence similarity), require a less vigorous validation. In such cases, it is more the specificity of the overall methodology, rather that of the individual probes that need to be validated (Wang *et al.*, 2002; Taroncher-Oldenburg *et al.*, 2003).

3. Data analysis

Microbial diagnostic microarray experiments usually involve the hybridization of a single sample per microarray (single colour experiment, as opposed to two-colour hybridizations typical for transcriptome analysis, employing competitive hybridization of a control sample vs the sample of interest; Schena, 2003). Fluorescence signals are normalized against

positive controls (targeting conserved regions, PCR primers applied or exogenous spiking DNA). Normalized signals for the individual probes are then typically rated as present or absent. This decision is usually made depending on an arbitrary signal intensity threshold, ideally reflecting both the overall limitation of the microarray platform and of the hybridization potential of the individual probes (Loy *et al.*, 2002; Bodrossy *et al.*, 2003; Lin *et al.*, 2004; Mitterer *et al.*, 2004; Nübel *et al.*, 2004; Peplies *et al.*, 2004). The complex hybridization patterns of MDM experiments are thus usually still translated manually into inventory lists of organisms present in the samples analysed. In an attempt to bridge the gap between data collection and analysis, a simple command line-based program was recently developed for diagnostic microarrays (Loy *et al.*, 2002). (An advanced graphical user interface-based version, ChipAnalyser, is under development; H. Meier; unpublished).

4. Specificity, quantification and sensitivity

The ultimate specificity of microarray technology is the discrimination between a fully complementary target and a nontarget differing in only a single nucleotide. Recently, various enzyme-assisted hybridization and signal amplification strategies have been reported promising improved discrimination of single mismatches located near the 3′ end of microarray probes (Mikhailovich *et al.*, 2001; Busti *et al.*, 2002; Gharizadeh *et al.*, 2003; Rudi *et al.*, 2003; Mitterer *et al.*, 2004) and enhancing the detection of low abundance microorganisms (Denef *et al.*, 2003), respectively.

The fundamental potential of microarrays to provide quantitative data on the abundance of the detected target in a sample is widely acknowledged. Adopting the two-colour hybridization approach from transcriptome microarray analysis, relative abundance of targets in a sample (labelled in colour one) can be measured by competitive hybridization with target mixtures of known concentrations (labelled in colour two) on the microarray (Rudi *et al.*, 2002; Bodrossy *et al.*, 2003). For single-colour hybridizations, a linear correlation between the signal intensity of a probe and the concentration of the respective target sequence has been observed for a certain range of target concentrations (Cho & Tiedje, 2002; Taroncher-Oldenburg *et al.*, 2003; Tiquia *et al.*, 2004). A semiquantitative comparison of similar community structures has been demonstrated, enabling the researcher to detect spatial and temporal changes in microbial community composition (Stralis-Pavese *et al.*, 2004). However, the slope and range of this linear relationship will vary among the different probes immobilized on a microarray because, as mentioned previously, different probes display different affinities to their targets.

Semiquantitative information can be obtained on the relative abundance of the microorganisms detected. This is especially useful when comparing similar samples for changes in the microbial community structure (e.g. temporal or spatial changes in soil microbial community structure). Absolute quantification is plagued by biases associated with, for example, PCR amplification, nucleic acid recovery, and different numbers of (marker) 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%. Some enzyme-based labelling methods (Busti *et al.*, 2002; Baner *et al.*, 2003; Gharizadeh *et al.*, 2003; Rudi *et al.*, 2003) have demonstrated the potential for an improved detection sensitivity (down to *c.* 0.1% of the total community targeted).

IV. Application of microarrays in the soil environment

As outlined above environmental, diagnostic microarrays can be divided into three classes: phylochips, FGAs and CGAs. Phylochips are based on the use of rRNA genes or other phylogenetic markers and they are usually applied in order to detect specific bacteria such as pathogens (Franke-Whittle *et al.*, 2005) in an ecosystem or to study diversity and the structure of microbial communities (Loy *et al.*, 2002; Loy *et al.*, 2004; Günther *et al.*, 2005). Phylochips, which address phylogenetic groups inevitably required for specific ecological processes, also yield substantial functional information on a particular ecosystem (Loy *et al.*, 2004). Functional gene arrays target genes encoding enzymes, which play key roles in various ecological processes such as nitrification, denitrification, nitrogen fixation, methane oxidation, sulphate reduction and the degradation of pollutants. They are particularly useful in addressing specific environmental processes and may be applied in order to reveal the community structure of microorganisms involved in these processes as well as addressing their activities. community genome arrays (CGAs) are based on whole genomes isolated from cultured microorganisms (Krause *et al.*, 2004; Wu *et al.*, 2004). However, they may be potentially be made from metagenomic environmental libraries or community genomes with reduced complexities (see below) and thereby would target also the uncultured microflora.

1. Phylochips for the soil environment

Phylogenetic oligonucleotide microarrays have been developed to study the diversity and community structure of certain phylogenetic groups in the environment. Recently, a microarray was designed to particularly address the genus *Kitasatospora* belonging to the actinomycetes (Günther *et al.*, 2005). *Kitasatospora*, which is closely related to the genus *Streptomyces*, is also known for the production of interesting secondary metabolites with new chemical structures and biological activities with high application potential. As the 16S/23S rRNA genes do not resolve close phylogenetic relationships, such as those between kitasatosporae and

streptomycetes, the ITS was used to design specific probes. The microarray contains 29 specific oligonucleotides and was applied to detect *Kitasatospora* in spiked soils and to identify members belonging to this genus in a forest soil (Günther *et al.*, 2005). The authors proposed the application of the microarray for an accelerated screening of soils for sorting already known species and further processing interesting samples (Günther *et al.*, 2005).

A 16S rRNA gene based oligonucleotide microarray consisting of 79 18-mer probes targeting different phylogenetic levels of the Betaproteobacteria order *Rhodocyclales* was developed (Loy *et al.*, 2005). Members of this order comprise a physiologically versatile group of bacteria, many of them responsible for the removal of anthropogenic compounds in the environment including *Azoarcus*, *Dechloromonas* and *Azospira*, which are known for their degrading capacities (Coates *et al.*, 1999; Achenbach *et al.*, 2001; Song *et al.*, 2001; Mechichi *et al.*, 2002). Other Rhodocyclales members such as *Azoarcus* species live in association with grasses, where they fix nitrogen (Reinhold-Hurek *et al.*, 1993; Engelhard *et al.*, 2000). The *Rhodocyclales* microarray has so far been applied in the diversity analysis of an activated sludge from an industrial wastewater treatment (Loy *et al.*, 2005), but may find future applications in the analysis of soil microbial populations. Furthermore, a prototype of a taxonomic 16S rRNA-based microarray has been developed, which contains 122 probes targeting bacteria at various taxonomic levels from phyla to species belonging mainly to the Alphaproteobacteria (Sanguin *et al.*, 2005). The array has been validated by using a range of bacterial strains as hybridization targets as well as by analysing *Agrobacterium* diversity in the rhizosphere of maize and by comparing microarray results with those obtained by the analysis of clone libraries. As Alphaproteobacteria play an important role in the interaction with plants, such a microarray might be very useful in understanding the ecology of plant-associated microorganisms, particularly if amended with probes for additional, relevant phyla.

The microbial community structure in compost determines greatly compost quality and different populations are involved in different processes at different stages during compost development. A microarray has been designed targeting the species usually encountered in compost (Franke-Whittle *et al.*, 2005). Furthermore, there are concerns regarding the use of composts on agricultural soils and the spread of human, animal and plant pathogens. The microarray developed therefore contains 12 probes with differing levels of specificity targeting actinomycetes and other organisms with significance as degraders in the composting process and 35 probes specific to pathogens and was suggested to offer potential for process monitoring, and the detection of pathogens as well as of beneficial microbes (Franke-Whittle *et al.*, 2005).

Several 16S rRNA gene based microarrays have been developed specifically to target important physiological processes such as sulphate reduction. Sulphate-reducing prokaryotes are of major importance mainly in marine systems (Jørgensen, 1982), however, they are also important in specialized terrestrial habitats that are frequently flooded, including rice fields (Wind *et al.*, 1999) and fens (Alewell & Novak, 2001; Loy *et al.*, 2004). Small *et al.* (2001) used the metal- and sulphate-reducing bacteria of the genera *Geobacter* and *Desulfovibrio* as a model system to make a prototype oligonucleotide microarray consisting of universal and species-specific probes. Loy *et al.* (2002) developed a microarray for the detection of all known lineages of sulphate-reducing prokaryotes, which consist of more than 130 described species so far, falling into four bacterial phyla and one archeal phylum. Sulphate reducers were thoroughly investigated in low-sulphate, acidic fens of a forested catchment in Germany, where the deposition of sulphur resulted from the combustion of soft coal (Loy *et al.*, 2004). Results confirmed the hypothesis that dissimilatory sulphate reduction is an on-going process in that environment. Microarray analysis which was supplemented with comparative analysis of 16S rRNA genes and dissimilatory (bi)sulphite reductase (*dsrAB*) genes indicated novel sulphate- or sulphite-reducing prokaryotes possibly adapted to low-sulphate habitats (Loy *et al.*, 2004).

Phylochips have been also applied in bioremediation research, although FGAs appear to have a higher potential to reveal information on degradation activities. Neufeld *et al.* (2006) prepared a library of hypervariable 16S rRNA gene sequences from a composite of uncontaminated soils and soils contaminated with hexachlorocyclohexane (HCH), and further selected the 100 most abundant types to be spotted on an array. Hybridization with PCR-amplified DNA from individual soil samples showed strong correlations of several probe signals with soil physicochemical parameters. Results demonstrated that probes targeting unknown Gammaproteobacteria and members of the genus *Sphingomonas* were associated with high HCH concentrations, implying the involvement of these bacteria in HCH degradation. Their results also showed good agreement between microarray and DNA fingerprinting data. Dendrograms based on Pearson correlations of DGGE fingerprints and microarray probe signals were highly similar, showing distinct clustering of contaminated and uncontaminated soil samples (Fig. 3; Neufeld *et al.*, 2006).

A new approach to study functional activities by using a phylochip was presented by Adamczyk *et al.* (2003). A small microarray consisting of 16S rRNA-based oligonucleotide probes targeting ammonia-oxidizing bacteria was used to identify cells that consume a ^{14}C-labelled substrate. In the isotope array approach, total RNA is isolated, fluorescently labelled and hybridized with the microarray and subsequently scanned for fluorescence as well as for radioactivity. The suitability of the approach was demonstrated for monitoring community composition and CO_2 fixation activity of ammonia-oxidizers in two nitrifying activated sludge samples (Adamczyk *et al.*, 2003).

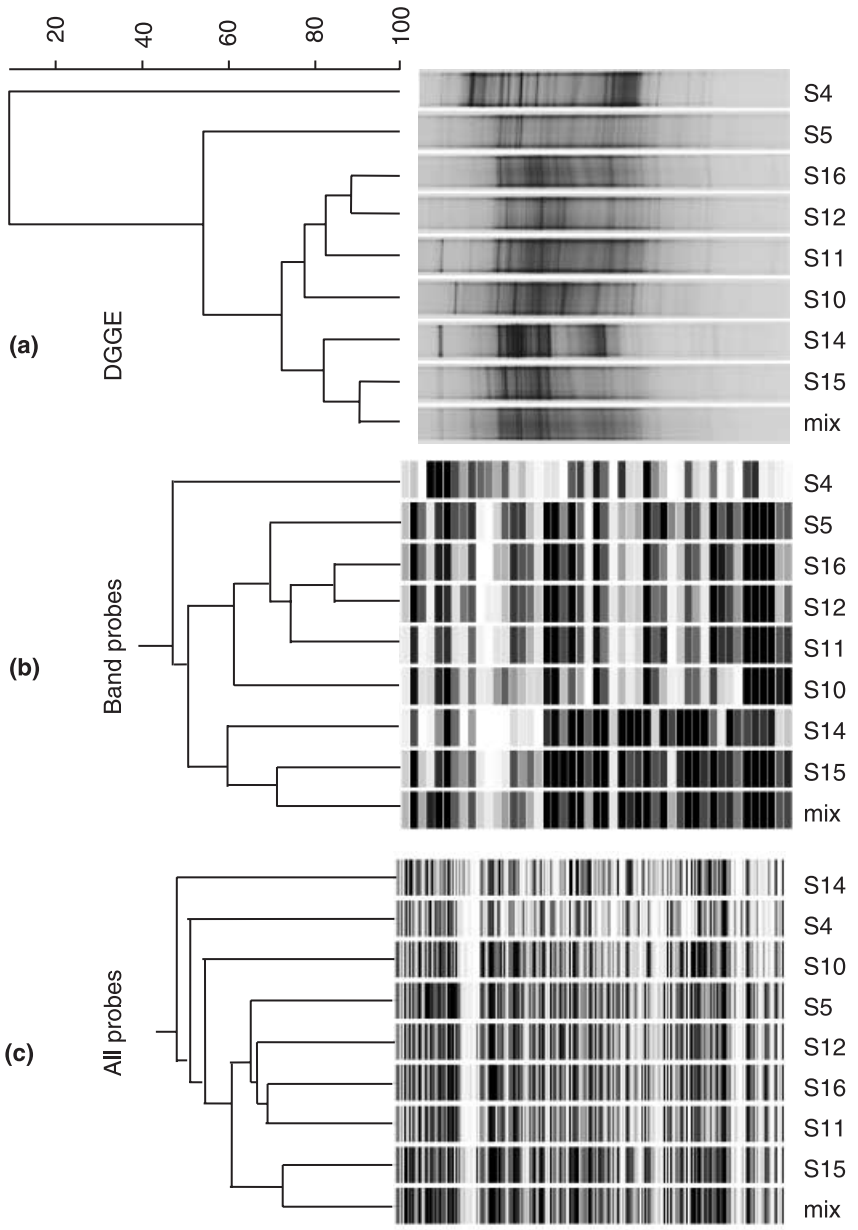

Fig. 3 Pearson correlation UPGMA dendrograms for the habitat-specific microarray and denaturating gradient gel electrophoresis (DGGE) analysis of microbial soil community compositions (Neufeld *et al*., 2006). Dendrograms for (a) DGGE fingerprints, (b) microarray signals for probes corresponding to DGGE bands and (c) microarray signals for all probes on the array. Microarray probe signal intensities are indicated with arbitrary shading. The darkest fill represents the most intense signal.

2. Microarrays targeting soil functional genes and activities

With the application of FGAs usually a well-defined, physiologically restricted group of bacteria is investigated. The gene encoding the particulate methane monooxygenase gene (*pmoA*) – the key enzyme in methane oxidation – was chosen for the development of a microarray to identify methanotrophs (Bodrossy *et al*., 2003), bacteria which are capable of utilizing methane as their sole source of carbon and energy. They therefore play an essential role in mitigating the greenhouse effect by metabolizing most of the methane produced, for example, in landfill sites. Understanding the factors influencing their diversity and activity is of high importance in order to adapt environmental remediation strategies for optimal methane oxidation. The improved *pmoA* microarray contains 68 (18- to 28-mer) probes targeting all known methanotrophs (including uncultivated members) as well as the related ammonium monooxygenase (*amoA*) genes of ammonium-oxidizing bacteria, and the phylogenetic resolution achieved was below the species level. It was applied in a lysimeter (mesocosm simulating landfill site conditions) experiment to study methane oxidation capacity and methanotroph diversity (Stralis-Pavese *et al*., 2004). This experiment simulated landfill sites with different plant vegetations and included also bare soil as well as biogas-free control lysimeters in order to investigate whether vegetation of landfill sites has the potential to improve

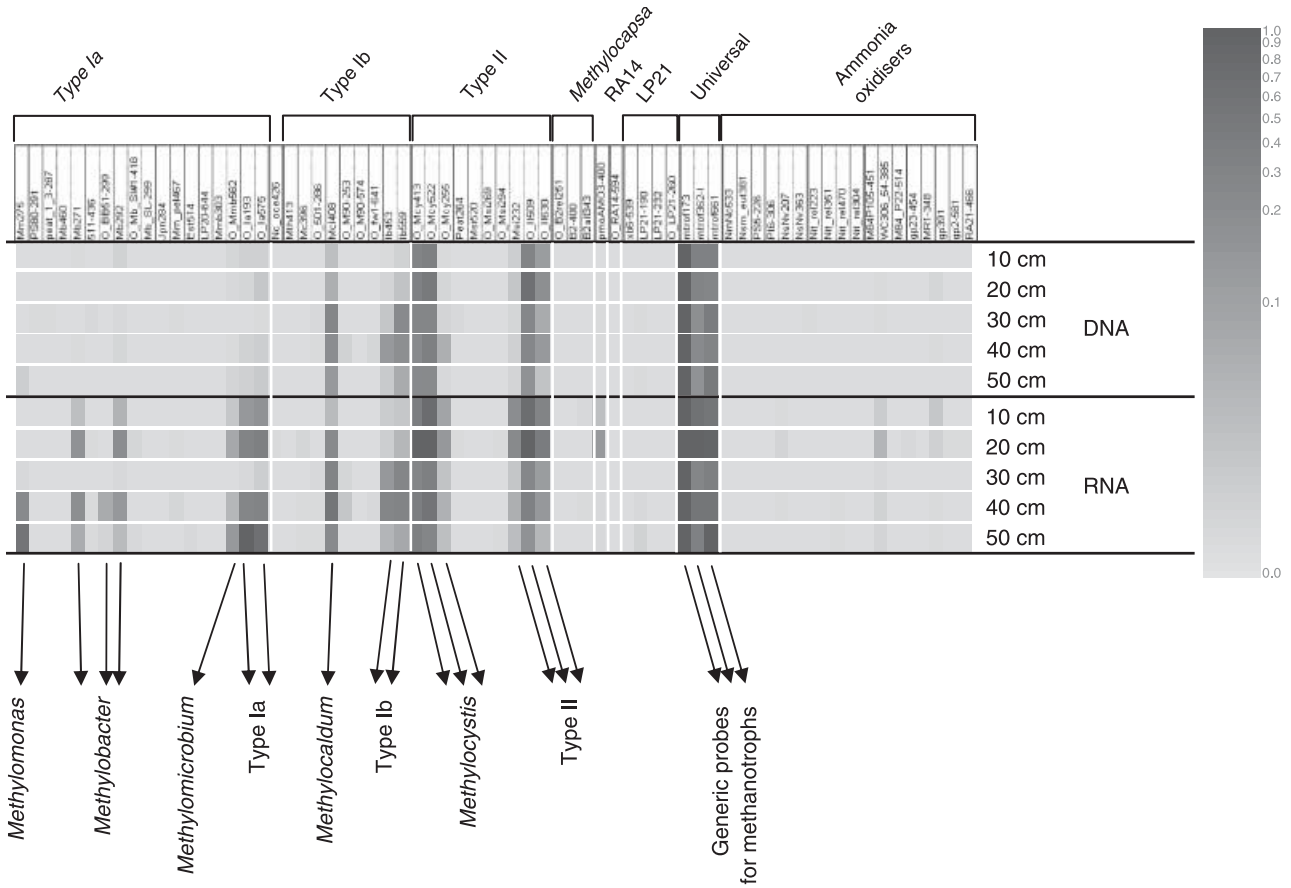

Fig. 4 Methanotroph community analyses with the *pmoA* oligonucleotide microarray using environmental DNA and mRNA as target.

methane oxidation. As lysimeters were also sampled at five different depths, the methanotroph community composition in more than 150 samples were thoroughly analysed, revealing a competitive advantage of type II methanotrophs under the conditions applied (Stralis-Pavese *et al.*, 2004). Although all vegetated lysimeters performed much better than the bare soil control, the best performance was identified in lysimeters where *Methylocystis* was dominating, indicating efficient methane oxidation by members of this genus. The *pmoA* microarray was also applied to analyse PCR products from mRNA transcripts in selected lysimeter samples detecting essentially similar type II methanotrophs; however, in addition a broad diversity of type Ia methanotrophs were identified, none of which had been detectable when the microarray analysis had been done from DNA (Bodrossy *et al.*, 2006) (Fig. 4). These results indicate that very different information can be obtained when gene transcripts are detected rather than the genes themselves. These results show that mRNA-based analyses may provide additional information on the composition and functioning of a microbial community, however, results should be treated with care. As mRNAs are very short-lived, expression analysis yields information on bacterial activities at the time point of sampling and transcript levels may be highly sensitive to altering environmental conditions, whereas DNA-based community analysis results are expected to be more stable but suffer from the limitation that cells are detected irrespective of their activity.

Various FGAs have been developed in order to address microorganisms and their genes involved in biogeochemical cycling (Wu *et al.*, 2001; Cho & Tiedje, 2002; Taroncher-Oldenburg *et al.*, 2003; Steward *et al.*, 2004; Tiquia *et al.*, 2004). The main metabolic activities addressed include nitrification (*amoA*), denitrification (*nirK* and *nirS*), nitrogen fixation (*nifH*) and in some cases methane oxidation (*pmoA*) as well as sulphite reduction (*dsrAB*), and these FGAs have been mainly developed to understand microbial ecology and biogeochemistry of aquatic systems (Taroncher-Oldenburg *et al.*, 2003; Jenkins *et al.*, 2004; Tiquia *et al.*, 2004). The same processes are highly important in soils, and therefore these arrays could provide an important basis for further development, however, the design of additional probes, which target soil microorganisms, might be required. A 70-mer long oligonucleotide FGA for the study of the nitrogen cycle, containing *nirS*, *nirK*, *nifH* and *amoA* probes, was developed (Taroncher-Oldenburg *et al.*, 2003). The specificity threshold

was 87%, enabling perfect signal separation between and differentiation of major lineages within the different gene families. Studies using the *nirS* set of 64 70-mer probes showed significant changes in the denitrifier community along a gradient of salinity, dissolved organic carbon and inorganic nitrogen. A related microarray was developed by Tiquia *et al.* (2004), which contained 763 50-mer probes targeting genes involved in nitrogen and sulphur cycling, and gene sequences with less than 86–90% similarity were discriminated. Initial environmental applications demonstrated that the microarray hybridized with DNAs obtained from marine sediments (Tiquia *et al.*, 2004). In order to study diazotroph diversity of a marine sediment, *nifH* gene fragments amplified from a clone library were spotted onto nylon membranes (Steward *et al.*, 2004), and the macroarray was applied to study the spatial variability of diazotroph communities in this sediment (Jenkins *et al.*, 2004).

A variety of microarrays has been developed for application in bioremediation studies. The most comprehensive array was developed by Rhee *et al.* (2004), which comprises 1662 unique and group-specific 50-mer probes targeting most of the genes and pathways known (at that time) to be involved in biodegradation and metal resistance. It was shown to differentiate sequences that had < 88% similarity. Its applicability was demonstrated in naphthalene-amended enrichment cultures as well as in microcosm experiments with soil containing polyaromatic hydrocarbons. Denef *et al.* (2003) constructed a microarray containing 45-mer and 70-mer probes targeting genes involved in the degradation of polychlorinated biphenyls (PCBs) and chlorobenzoate. Several parameters such as probe modification, microarray type and signal amplification were evaluated in order to achieve a detection signal of 1% of the total community. The applicability of the microarray and its detection limit were verified in a microcosm experiment with contaminated sediment, which was inoculated with a PCB-dechlorinating community (Denef *et al.*, 2003). Furthermore, a prototype microarray consisting of 64 gene fragment probes targeting various known catabolic and metabolic microbial genes was developed and tested for expression analysis of these genes in microbial communities (Dennis *et al.*, 2003). Induction of two of five 2,4-dichlorophenoxy acetic acid (2,4-D) catabolic genes (*tfdA* and *tfdC*) from populations of a degrading inoculant strain as low as 10^5 cells ml^{-1} was clearly detected against a background of 10^8 cells ml^{-1} (Dennis *et al.*, 2003).

3. Whole-genome microarrays in soil microbial ecology

Whole genome-based information is widely used to analyse gene expression in both prokaryotes and eukaryotes and to understand gene regulation (DeRisi *et al.*, 1997; Wodicka *et al.*, 1997; Ye *et al.*, 2000). Furthermore, whole-genome arrays have been applied for profiling individual organisms and to identify molecular markers correlating with specific phenotypic traits (Chee *et al.*, 1996; Wenzl *et al.*, 2004). Whole-genome microarrays have proven particularly useful in comparative genomics identifying genomic differences between closely related species or even strains (Salama *et al.*, 2000; Dong *et al.*, 2001; Kato-Maeda *et al.*, 2001; Murray *et al.*, 2001). Ongoing sequencing efforts in combination with comparative genomics analyses will substantially help in the identification of novel genes which tightly correlate with a particular phenotype such as degradation activities or heavy metal resistance. Microbial genomic hybridization will also provide useful in further understanding microbial speciation and taxonomic relationships.

Recently, a prototype whole-genome community array was constructed and evaluated with environmental samples (Wu *et al.*, 2004). The microarray contained whole genomic DNA (probes) from 32 type strains and from 35 environmental isolates and when applied for the analysis of spiked soil samples showed a detection limit of approximately 2.5×10^5 cells of an individual target genome in the presence of other bacterial cells. It was further applied for profiling microbial communities in surface soils, river sediments and marine sediments and as the CGA prototype revealed differences in these environments, it was suggested that CGAs have potential to be applied in the detection and identification of microorganisms in environmental samples (Wu *et al.*, 2004). Similarly, Krause *et al.* (2004) constructed a CGA based on 96 bacterial genomes, which was applied to study the effect of a diet containing *Acacia angustissima*, which is rich in nonprotein amino acids and tannins potentially toxic to animals, on rumen microbial communities.

Whole-genome microarrays are a promising tool for investigating strain-specific characteristics and genes that cannot be targeted easily by other approaches. However, they are usually developed from cultivated microorganisms, which only represent a small fraction of microbial communities. Diversity Arrays Technology (DArT) is an innovative, cultivation-independent and sequence information-independent approach to the whole-genome-based analysis of microbial communities. It uses an array of individualized clones of a metagenomic representation (e.g. amplifiable restriction fragments) prepared from a pool of DNA samples similar to those to be analysed. Representations derived from individual samples are hybridized to the array to identify informative array features that discriminate samples. It does not require any previous knowledge of functional relationships and has the potential to identify diagnostic sequence motifs that technologies focusing on 'functional genes' may miss.

Originally, DArT was developed for whole-genome profiling of a range of plant species (Jaccoud *et al.*, 2001; Lezar *et al.*, 2004; Wenzl *et al.*, 2004; Kilian *et al.*, 2005; Wittenberg *et al.*, 2005; Xia *et al.*, 2005) but has also been used to genotype individual microorganisms such as fungal plant pathogens (A.H.J. Wittenberg *et al.* unpublished). In addition, the initial 'proof of concept' paper reported the ability of

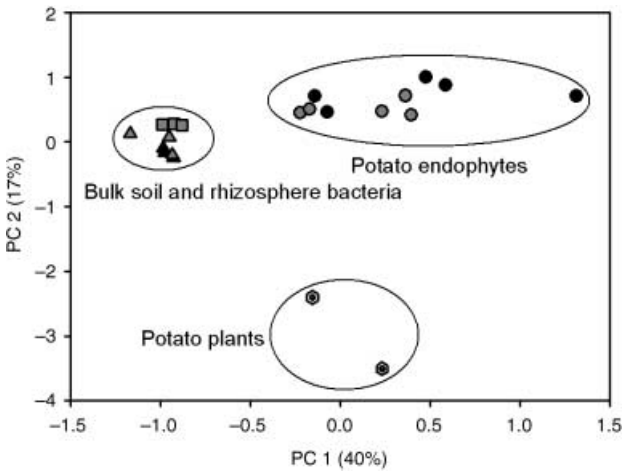

Fig. 5 Principal components analysis (PCA) of the hybridization data obtained with a Diversity Arrays Technology (DArT) array developed from a pool of endophytic microbial communities of potato plants. The array was hybridized with metagenomic representations of different endophyte, rhizosphere and bulk-soil microbial communities collected during an experiment in which potato plants were grown on a variety of agricultural soils. Circles, endophyte communities; triangles, rhizosphere communities; squares, bulk-soil communities; hexagons, plant DNA (potato variety Merkur). Closed symbols represent samples derived from trials with potato plants inoculated with the pathogen *Erwinia carotovora*.

DArTs to detect microbial variation in complex DNA mixtures (Jaccoud *et al.*, 2001). A pilot study was performed to explore the potential of expanding the technology to the analysis of microbial communities. Endophytic microorganisms of potato plants were selected as an example for microbial communities of intermediate complexity. A small number of restriction enzyme-based methods were tested to prepare metagenomic representations with a sequence complexity that is suitable for DArT assays. Figure 5 shows a test of an array developed with one of these methods from the pooled metagenomes of endophytic communities. The array discriminated the endophyte metagenomes against DNA preparations of soil and rhizosphere microbial communities and potato plants (a potential contaminant in the endophyte preparations) and revealed differences in the composition of the endophytic communities. Experience with DArT analyses of fungi and plants suggests that an optimization of the method(s) used to prepare metagenomic representations is likely to increase the discriminatory power of DArT arrays for microbial communities.

V. Conclusions

Many, often conflicting aspects and goals have to be considered and integrated during microarray design and application in order to fulfil the potential of MDMs as high-throughput screening tools for routine analysis. The key to a reliable MDM, and consequently to meaningful results, is rigorous *in silico* and *in vitro* performance testing. An additional point that has to be taken into consideration is that microarray-based analysis may be biased by the kind of DNA isolation method applied or by PCR amplification procedures.

Technologies used for MDMs are highly diverse but PCR amplification, followed by conventional fluorescence labelling is frequently used. Specificity is then achieved by the hybridization step. Depending on the marker genes applied, current MDMs can provide resolution at various taxonomic levels down to species or even strain level. Ribosomal RNA genes are often used as marker genes, however, they confer only limited phylogenetic resolution, and therefore rRNA genes are of limited use in many applications. Sometimes rRNA genes can be used as a marker for functionally related organisms, but in many cases functional information is not obtained by using rRNA-based microarrays. Alternative, higher resolution phylogenetic markers are thus beginning to be applied, with short oligonucleotide probes, used in a hierarchical and parallel manner, being the preferred choice.

The prediction of the hybridization behaviour of the probes is still rudimentary and requires improvement. This can be achieved via input from bioinformatics and the establishment of datasets on array-specific effects such as immobilization and steric hindrance.

Large databases, comparable in size to that of the 16S rRNA databases, are critically needed for high-resolution phylogenetic markers. Such markers will drastically improve the applicability of MDMs for soil microbial analysis but also for other fields including clinical and food microbiology, and epidemiology. If, in such databases, sequence information is linked to functional activities or clinical traits (e.g. biodegradative abilities, involvement in nutrient cycling, pathogenicity, host specificity, antibiotic resistance and geographic origin), MDM-based detection will serve the additional purpose of providing a prediction of these functions, and at least on some of them at a given level of certainty. Similarly, the build up of databases containing information on key enzymes/genes involved in important soil functions would be extremely helpful for the development of MDMs to be applied for monitoring biogeochemical processes or addressing other important functional activities of microorganims in soil. This implies that relevant genes from yet uncultivated microbes have to be available, here metagenomic approaches will greatly contribute.

In the process of understanding the functioning of microbial communities in soil, microarrays greatly contribute by their application as a high-throughput analytical tool for monitoring population changes in detail, both at a temporary and spatial scale. Coupling such a thorough community analysis with data on physical and chemical processes will certainly contribute to a better understanding of the soil ecosysem. In many cases, the design of microarrays specific for a particular soil environment/site will be useful in order to address important microbial players in that environment

(Neufeld *et al.*, 2006). This will particularly apply for functional genes, which frequently show a rather low degree of conservation and are highly adapted to that environment/site. Finally, the application of microarray technology is not restricted to identification of genes/microorganisms in the environment, it also has great potential for ecophysiological characterization of microbial populations. Transcript analysis with microarrays must to be treated with care because of the rapid changes in expression that may occur. However, when used at high throughput, this may lead to novel functional information not available by DNA-based analysis.

Acknowledgements

Related work at Seibersdorf research was funded by the Fonds zur Förderung der wissenschaftlichen Forschung, Austria (P15044). The DArT pilot study was funded through an OECD-sponsored travel fellowship to Evelyn Hackl to visit DArT P/L. L.B. is indebted to Fodor Szilvia for her support and fresh, independent views and ideas.

References

Achenbach LA, Michaelidou U, Bruce RA, Fryman J, Coates JD. 2001. *Dechloromonas agitata* gen. nov., sp. nov. and *Dechlorosoma suillum* gen. nov., sp. nov., two novel environmentally dominant (per)chlorate-reducing bacteria and their phylogenetic position. *International Journal of Systematic and Evolutionary Microbiology* **51**: 527–533.

Adamczyk J, Hesselsoe M, Iversen N, Horn M, Lehner A, Nielsen PH, Schloter M, Roslev P, Wagner M. 2003. The isotope array, a new tool that employs substrate-mediated labeling of rRNA for determination of microbial community structure and function. *Applied and Environmental Microbiology* **69**: 6875–6887.

Al Khaldi SF, Myers KM, Rasooly A, Chizhikov V. 2004. Genotyping of *Clostridium perfringens* toxins using multiple oligonucleotide microarray hybridization. *Molecular Cell Probes* **18**: 359–367.

Alewell C, Novak M. 2001. Spotting zones of dissimilatory sulfate reduction in a forested catchment: the 34S–35S approach. *Environmental Pollution* **112**: 369–377.

Alexander M. 1977. *Introduction to soil microbiology*, 2nd edn. New York, NY, USA: Wiley.

Amann R, Ludwig W, Schleifer KH. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiology Reviews* **59**: 143–169.

Anthony RM, Brown TJ, French GL. 2000. Rapid diagnosis of bacteremia by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. *Journal of Clinical Microbiology* **38**: 781–788.

Anthony RM, Schuitema ARJ, Oskam L, Klatser PR. 2005. Direct detection of *Staphylococcus aureus* mRNA using a flow through microarray. *Journal of Microbiological Methods* **60**: 47–54.

Bae JW, Rhee SK, Nam YD, Park YH. 2005. Generation of subspecies level-specific microbial diagnostic microarrays using genes amplified from subtractive suppression hybridization as microarray probes. *Nucleic Acids Research* **33**: e113.

Baldauf SL, Palmer JD, Doolittle WF. 1996. The root of the universal tree and the origin of eukaryotes based on elongation factor phylogeny. *Proceedings of the National Academy of Sciences, USA* **93**: 7749–7754.

Baner J, Isaksson A, Waldenstrom E, Jarvius J, Landegren U, Nilsson M. 2003. Parallel gene analysis with allele-specific padlock probes and tag microarrays. *Nucleic Acids Research* **31**: e103.

Behr T, Koob C, Schedl M, Mehlen A, Meier H, Knopp D, Frahm E, Obst U, Schleifer K, Niessner R, Ludwig W. 2000. A nested array of rRNA targeted probes for the detection and identification of enterococci by reverse hybridization. *Systematic and Applied Microbiology* **23**: 563–572.

Bodrossy L, Sessitsch A. 2004. Oligonucleotid microarrays in microbial diagnostics. *Current Opinion Microbiology* **7**: 245–254.

Bodrossy L, Stralis-Pavese N, Murrell JC, Radajewski S, Weilharter A, Sessitsch A. 2003. Development and validation of a diagnostic microbial microarray for methanotrophs. *Environmental Microbiology* **5**: 566–582.

Bodrossy L, Stralis-Pavese N, Konrad-Köszler M, Weilharter A, Reichenauer T, Schöfer D, Sessitsch A. 2006. mRNA-based parallel detection of active methanotroph populations using a diagnostic microarray. *Applied and Environmental Microbiology* **72**: 1672–1676.

Bonch-Osmolovskaya EA, Miroshnichenko ML, Lebedinsky AV, Chernyh NA, Nazina TN, Ivoilov VS, Belyaev SS, Boulygina ES, Lysov YP, Perov AN, Mirzabekov AD, Hippe H, Stackebrandt E, L'Haridon S, Jeanthon C. 2003. Radioisotopic, culture-based, and oligonucleotide microchip analyses of thermophilic microbial communities in a continental high-temperature petroleum reservoir. *Applied and Environmental Microbiology* **69**: 6143–6151.

Brisse S, Verhoef J. 2001. Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, *gyrA* and *parC* genes sequencing and automated ribotyping. *International Journal of Systematic and Evolutionary Microbiology* **51**: 915–924.

Busti E, Bordoni R, Castiglioni B, Monciardini P, Sosio M, Donadio S, Consolandi C, Bernardi LR, Battaglia C, De Bellis G. 2002. Bacterial discrimination by means of a universal array approach mediated by LDR (ligase detection reaction). *BMC Microbiology* **2**: 27.

Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SPA. 1996. Accessing genetic information with high-density DNA arrays. *Science* **274**: 610–614.

Cho JC, Tiedje JM. 2002. Quantitative detection of microbial genes by using DNA microarrays. *Applied and Environmental Microbiology* **68**: 1425–1430.

Clewley JP. 2004. A role for arrays in clinical virology: fact or fiction? *Journal of Clinical Virology* **29**: 2–12.

Coates JD, Michaelidou U, Bruce RA, O'Connor SM, Crespi JN, Achenbach LA. 1999. Ubiquity and diversity of dissimilatory (per) chlorate-reducing bacteria. *Applied and Environmental Microbiology* **65**: 5234–5241.

Cole JR, Chai B, Marsh TL, Farris RJ, Wang Q, Kulam SA, Chandra S, McGarrell DM, Schmidt TM, Garrity GM, Tiedje JM. 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Research* **31**: 442–443.

Cook KL, Layton AC, Dionisi HM, Fleming JT, Sayler GS. 2004. Evaluation of a plasmid-based 16S–23S rDNA intergenic spacer region array for analysis of microbial diversity in industrial wastewater. *Journal of Microbiological Methods* **57**: 79–93.

Dahllöf I, Baillie H, Kjelleberg S. 2000. *rpoB*-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. *Applied and Environmental Microbiology* **66**: 3376–3380.

Denef VJ, Park J, Rodrigues JL, Tsoi TV, Hashsham SA, Tiedje JM. 2003. Validation of a more sensitive method for using spotted oligonucleotide DNA microarrays for functional genomics studies on bacterial communities. *Environmental Microbiology* **5**: 933–943.

Dennis P, Edwards EA, Liss SN, Fulthorpe R. 2003. Monitoring gene expression in mixed microbial communities by using DNA microarrays. *Applied and Environmental Microbiology* **69**: 769–778.

DeRisi JL, Iyer VR, Brown PO. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**: 680–686.

DeSantis TZ, Stone CE, Murray SR, Moberg JP, Andersen GL. 2005. Rapid quantification and taxonomic classification of environmental DNA from both prokaryotic and eukaryotic origins using a microarray. *FEMS Microbiology Letters* **245**: 271–278.

Dong YM, Glasner JD, Blattner FR, Triplett EW. 2001. Genomic interspecies microarray hybridization: rapid discovery of three thousand genes in the maize endophyte, *Klebsiella pneumoniae* 342, by microarray hybridization with *Escherichia coli* K-12 open reading frames. *Applied and Environmental Microbiology* **67**: 1911–1921.

Drancourt M, Roux V, Fournier PE, Raoult D. 2004. *rpoB* gene sequence-based identification of aerobic Gram-positive cocci of the genera *Streptococcus, Enterococcus, Gemella, Abiotrophia*, and *Granulicatella*. *Journal of Clinical Microbiology* **42**: 497–504.

Druzhinina IS, Kopchinskiy AG, Komon M, Bissett J, Szakacs G, Kubicek CP. 2005. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genetics and Biology* **42**: 813–828.

Dunbar J, Takala S, Barns SM, Davis JA, Kuske CR. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Applied and Environmental Microbiology* **65**: 1662–1669.

Engelhard M, Hurek T, Reinhold-Hurek B. 2000. Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp. in wild rice species and land races of *Oryza sativa* in comparison with modern races. *Environmental Microbiology* **2**: 131–141.

Franke-Whittle I, Klammer S, Insam H. 2005. Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. *Journal of Microbiological Methods* **62**: 37–56.

Gharizadeh B, Kaller M, Nyren P, Andersson A, Uhlen M, Lundeberg J, Ahmadian A. 2003. Viral and microbial genotyping by a combination of multiplex competitive hybridization and specific extension followed by hybridization to generic tag arrays. *Nucleic Acids Research* **31**: e146.

Greene EA, Voordouw G. 2003. Analysis of environmental microbial communities by reverse sample genome probing. *Journal of Microbiological Methods* **53**: 211–219.

Grundmann GL, Normand P. 2000. Microscale diversity of the genus *Nitrobacter* in soil on the basis of analysis of genes encoding rRNA. *Applied and Environmental Microbiology* **66**: 4543–4546.

Günther S, Groth I, Grabley S, Munder T. 2005. Design and evaluation of an oligonucleotide microarrays for the detection of different species of the genus *Kitasatospora*. *Journal of Microbiological Methods* **65**: 226–236.

Guschin DY, Mobarry BK, Proudnikov D, Stahl DA, Rittmann BE, Mirzabekov AD. 1997. Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. *Applied and Environmental Microbiology* **63**: 2397–2402.

Hackl E, Zechmeister-Boltenstern S, Bodrossy L, Sessitsch A. 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Applied and Environmental Microbiology* **70**: 5057–5065.

Handelsman J. 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews* **68**: 669–685.

Hashsham SA, Wick LM, Rouillard JM, Gulari E, Tiedje JM. 2004. Potential of DNA microarrays for developing parallel detection tools (PDTs) for microorganisms relevant to biodefense and related research needs. *Biosensors and Bioelectronics* **20**: 668–683.

He Z, Wu L, Fields MW, Zhou J. 2005. Use of microarrays with different probe sizes for monitoring gene expression. *Applied and Environmental Microbiology* **71**: 5154–5162.

Huang S, Wang Y, Chen P, Qian HR, Yeo A, Bemis K. 2004. SUM: a new way to incorporate mismatch probe measurements. *Genomics* **84**: 767–777.

Hughes TR, Mao M, Jones AR, Burchard J, Marton MJ, Shannon KW, Lefkowitz SM, Ziman M, Schelter JM, Meyer MR, Kobayashi S, Davis C, Dai H, He YD, Stephaniants SB, Cavet G, Walker WL, West A, Coffey E, Shoemaker DD, Stoughton R, Blanchard AP, Friend SH, Linsley PS. 2001. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nature Biotechnology* **19**: 342–347.

Jaccoud D, Peng K, Feinstein D, Kilian A. 2001. Diversity Arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research* **29**: e25.

Jacobs KA, Rudersdorf R, Neill SD, Dougherty JP, Brown EL, Fritsch EF. 1988. The thermal stability of oligonucleotide duplexes is sequence independent in tetraalkylammonium salt solutions: application to identifying recombinant DNA clones. *Nucleic Acids Research* **16**: 4637–4650.

Jenkins BD, Steward GF, Short SM, Ward BB, Zehr JP. 2004. Fingerprinting diazotroph communities in the Chesapeake bay by using a DNA macroarray. *Applied and Environmental Microbiology* **70**: 1767–1776.

Jørgensen BB. 1982. Mineralization of organic matter in the sea-bed – the role of sulphate reduction. *Nature* **296**: 643–645.

Joseph SJ, Hugenholtz P, Sangvan P, Osborne CA, Janssen PH. 2003. Laboratory cultivation of widespread and previously uncultured soil bacteria. *Applied and Environmental Microbiology* **69**: 7210–7215.

Kaiser O, Pühler A, Selbitschka W. 2001. Phylogenetic analysis of microbial diversity in the rhizoplane of oilseed rape (*Brassica napus* cv. West) employing cultivation-dependent and cultivation-independent approaches. *Microbial Ecology* **42**: 136–149.

Kakinuma K, Fukushima M, Kawaguchi R. 2003. Detection and identification of *Escherichia coli*, *Shigella*, and *Salmonella* by microarrays using the *gyrB* gene. *Biotechnology and Bioengineering* **83**: 721–728.

Kane MD, Jatkoe TA, Stumpf CR, Lu J, Thomas JD, Madore SJ. 2000. Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. *Nucleic Acids Research* **28**: 4552–4557.

Kato-Maeda M, Rhee JT, Gingeras TR, Salamon H, Drenkow J, Smittipat N, Small PM. 2001. Comparing genomes within the species *Mycobacterium tuberculosis*. *Genome Research* **11**: 547–554.

Kilian A, Huttner E, Wenzl P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska Cayla C, Patarapuwadol S, Xia L, Yang S, Thomson B. 2005. The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In: Tuberosa Royal, Phillips RL, Gale M, eds. *Proceedings of the International Congress 'in the Wake of the Double Helix: from the Green Revolution to the Gene Revolution': 27–31 May 2003. Bologna, Italy: Avenue Media*, 443–461.

Knief C, Vanitchung S, Harvey NW, Conrad R, Dunfield PF, Chidthaisong A. 2005. Diversity of methanotrophic bacteria in tropical upland soils under different land uses. *Applied Environmental Microbiology* **71**: 2005.

Kostic T, Weilharter A, Sessitsch A, Bodrossy L. 2005. High-sensitivity, polymerase chain reaction-free detection of microorganisms and their functional genes using 70-mer oligonucleotide diagnostic microarray. *Analytical Biochemistry* **346**: 333–335.

Krause DO, Smith WJM, McSweeney CS. 2004. Use of community genome arrays (CGAs) to assess the effects of *Acacia angustissima* on rumen ecology. *Microbiology* **150**: 2899–2909.

Kuske CR, Ticknor LO, Miller ME, Dunbar JM, Davis JA, Barns SM, Belnap J. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* **68**: 1854–1863.

Lee I, Dombkowski AA, Athey BD. 2004. Guidelines for incorporating non-perfectly matched oligonucleotides into target-specific hybridization probes for a DNA microarray. *Nucleic Acids Research* **32**: 681–690.

Lehner A, Loy A, Behr T, Gaenge H, Ludwig W, Wagner M, Schleifer KH. 2005. Oligonucleotide microarray for identification of *Enterococcus* species. *FEMS Microbiology Letters* **246**: 133–142.

Leinberger DM, Schumacher U, Autenrieth IB, Bachmann TT. 2005. Development of a DNA microarray for detection and identification of fungal pathogens involved in invasive mycoses. *Journal of Clinical Microbiology* **43**: 4943–4953.

Letowski J, Brousseau R, Masson L. 2004. Designing better probes: effect of probe size, mismatch position and number on hybridization in DNA

oligonucleotide microarrays. *Journal of Microbiological Methods* **57**: 269–278.

Lezar S, Myburg AA, Berger DK, Wingfield MJ, Wingfield BD. 2004. Development and assessment of microarray-based DNA fingerprinting in *Eucalyptus grandis*. *Theoretical and Applied Genetics* **109**: 1329–1336.

Li X, He Z, Zhou J. 2005. Selection of optimal oligonucleotide probes for microarrays using multiple criteria, global alignment and parameter estimation. *Nucleic Acids Research* **33**: 6114–6123.

Lin B, Vora GJ, Thach D, Walter E, Metzgar D, Tibbetts C, Stenger DA. 2004. Use of oligonucleotide microarrays for rapid detection and serotyping of acute respiratory disease-associated adenoviruses. *Journal of Clinical Microbiology* **42**: 3232–3239.

Lipson DA, Wilson Richard F, Oechel Walter C. 2005. Effects of elevated atmospheric CO_2 on soil microbial biomass, activity, and diversity in a chaparral ecosystem. *Applied and Environmental Microbiology* **71**: 8573–8580.

Liu WT, Mirzabekov AD, Stahl DA. 2001. Optimization of an oligonucleotide microchip for microbial identification studies: a non-equilibrium dissociation approach. *Environmental Microbiology* **3**: 619–629.

Lloyd AT, Sharp PM. 1993. Evolution of the *recA* gene and the molecular phylogeny of bacteria. *Journal of Molecular Evolution* **37**: 399–407.

Loy A, Bodrossy L. 2006. Highly parallel microbial diagnostics using oligonucleotide microarrays. *Clinica Chimica Acta* **363**: 106–119.

Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer K-H, Wagner M. 2002. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulphate-reducing prokaryotes in the environment. *Applied and Environmental Microbiology* **68**: 5064–5081.

Loy A, Horn M, Wagner M. 2003. probeBase: an online resource for rRNA-targeted oligonucleotide probes. *Nucleic Acids Research* **31**: 514–516.

Loy A, Küsel K, Lehner A, Drake HL, Wagner M. 2004. Microarray and functional gene analyses of sulphate-reducing prokaryotes in low-sulfate, acidic fens reveal coocurrence of recognized genera and novel lineages. *Applied and Environmental Microbiology* **70**: 6998–7009.

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*'. *Applied and Environmental Microbiology* **71**: 1373–1386.

Ludwig W, Schleifer KH. 1999. Phylogeny of bacteria beyond the 16S rRNA standard. *ASM News* **65**: 1515–1521.

Ludwig W, Neumaier J, Klugbauer N, Brockmann E, Roller C, Jilg S, Reetz K, Schachtner I, Ludvigsen A, Bachleitner M, Fisher U, Schleifer KH. 1993. Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase beta-subunit genes. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology* **64**: 285–305.

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 AW, 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 KH. 2004. ARB: a software environment for sequence data. *Nucleic Acids Research* **32**: 1363–1371.

Maskos U, Southern EM. 1992. Parallel analysis of oligodeoxyribonucleotide (oligonucleotide) interactions. I. Analysis of factors influencing oligonucleotide duplex formation. *Nucleic Acids Research* **20**: 1675–1678.

Mechichi T, Stackebrandt E, Gad'on N, Fuchs G. 2002. Phylogenetic and metabolic diversity of bacteria degrading aromatic compounds under denitrifying conditions, and description of *Thauera phenylacetica* sp. nov., *Thauera aminoaromatica* sp. nov. and *Azoarcus buckelii* sp. nov. *Archives Microbiology* **178**: 26–35.

Mikhailovich V, Lapa S, Gryadunov D, Sobolev A, Strizhkov B, Chernyh N, Skotnikova O, Irtuganova O, Moroz A, Litvinov V, Vladimirskii M, Perelman M, Chernousova L, Erokhin V, Zasedatelev A, Mirzabekov A. 2001. Identification of rifampicin-resistant *Mycobacterium tuberculosis* strains by hybridization, PCR, and ligase detection reaction on oligonucleotide microchips. *Journal of Clinical Microbiology* **39**: 2531–2540.

Mitterer G, Huber M, Leidinger E, Kirisits C, Lubitz W, Mueller MW, Schmidt WM. 2004. Microarray-based identification of bacteria in clinical samples by solid-phase PCR amplification of 23S ribosomal DNA sequences. *Journal of Clinical Microbiology* **42**: 1048–1057.

Mollet C, Drancourt M, Raoult D. 1997. *rpoB* sequence analysis as a novel basis for bacterial identification. *Molecular Microbiology* **26**: 1005–1011.

Murray AE, Lies D, Li G, Nealson K, Zhou J, Tiedje JM. 2001. DNA/DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes. *Proceedings of the National Academy of Sciences, USA* **98**: 9853–9858.

Neufeld JD, Mohn WW, de Lorenzo V. 2006. Composition of microbial communities in hexachlorocyclohexane (HCH) contaminated soils from Spain revealed with a habitat-specific microarray. *Environmental Microbiology* **8**: 126–140.

Nicolaisen M, Justesen AF, Thrane U, Skouboe P, Holmstrom K. 2005. An oligonucleotide microarray for the identification and differentiation of trichothecene producing and non-producing *Fusarium* species occurring on cereal grain. *Journal of Microbiological Methods* **62**: 57–69.

Nübel U, Schmidt PM, Reiss E, Bier F, Beyer W, Naumann D. 2004. Oligonucleotide microarrays for identification of *Bacillus anthracis* based on intergenic transcribed spacers in ribosomal DNA. *FEMS Microbiology Letters* **240**: 215–223.

Øvreås L, Torsvik V. 1998. Microbial diversity and community structure in two different agricultural soil communities. *Microbial Ecology* **36**: 303–315.

Peplies J, Lau SC, Pernthaler J, Amann R, Glockner FO. 2004. Application and validation of DNA microarrays for the 16S rRNA-based analysis of marine bacterioplankton. *Environmental Microbiology* **6**: 638–645.

Reinhold-Hurek B, Hurek T, Gillis M, Hoste M, Vancanneyt M, Kersters K, De Ley J. 1993. *Azoarcus* gen. nov., nitrogen fixing *Proteobacteria* associated with roots of kallar grass (*Leptochloa fusca* (L.) Kunth), and description of two species, *Azoarcus indigens* sp. nov. and *Azoarcus communis* sp. nov. *International Journal of Systematic Bacteriology* **43**: 574–584.

Rhee S-K, Liu X, Wu L, Chong SC, Wan X, Zhou J. 2004. Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. *Applied and Environmental Microbiology* **70**: 4303–4317.

Rudi K, Treimo J, Moen B, Rud I, Vegarud G. 2002. Internal controls for normalizing DNA arrays. *Bio/Techniques* **33**: 496,498,500.

Rudi K, Treimo J, Nissen H, Vegarud G. 2003. Protocols for 16S rDNA array analyses of microbial communities by sequence-specific labeling of DNA probes. *Scientific World Journal* **3**: 578–584.

Salama N, Guillemin K, McDaniel TK, Sherlock G, Tompkins L, Falkow S. 2000. A whole genome microarray reveals genetic diversity among *Helicobacter pylori* strains. *Proceedings of the National Academy of Sciences, USA* **97**: 14688–14673.

Sanguin H, Herrera A, Oger-Desfeux C, Dechesne A, Simonet P, Navarro E, Vogel TM, Moenne-Loccoz Y, Nesme X, Grundmann GL. 2005. Development and validation of a prototype 16S rRNA-based taxonomic microarray for *Alphaproteobacteria*. *Environmental Microbiology* **8**: 289–307.

Sangvan P, Kovac S, Davis KER, Sait M, Janssen PH. 2005. Detection and cultivation of soil verrucomicrobia. *Applied and Environmental Microbiology* **71**: 8402–8410.

Schena M. 2003. *Microarray analysis*. Hoboken, Canada: John Wiley and Sons, Inc.

Sergeev N, Volokhov D, Chizhikov V, Rasooly A. 2004. Simultaneous analysis of multiple staphylococcal enterotoxin genes by an oligonucleotide microarray assay. *Journal of Clinical Microbiology* **42**: 2134–2143.

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E. 2001. Microbial population structures in soil particle size fractions of a long-term

fertilizer experiment. *Applied and Environmental Microbiology* **67**: 4215–4224.

Small J, Call DR, Brockman FJ, Straub TM, Chandler DP. 2001. Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. *Applied and Environmental Microbiology* **67**: 4708–4716.

Smit E, Leeflang P, Gommans S, van den Broek J, van Mil S, Wernars K. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Applied and Environmental Microbiology* **67**: 2284–2291.

Song B, Palleroni NJ, Kerkhof J, Häggblom MM. 2001. Characterization of halobenzoate-degrading denitrifying *Azoarcus* and *Thauera* isolates and description of *Thauera chlorobenzoica* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **51**: 589–602.

Stenger D, Andreadis J, Voraa G, Pancrazio J. 2002. Potential applications of DNA microarrays in biodefense-related diagnostics. *Current Opinion Biotechnology* **13**: 208–212.

Stevenson BS, Eichorst SA, Wertz TW, Schmidt TM, Breznak JA. 2004. New strategies for cultivation of previously uncultured microbes. *Applied and Environmental Microbiology* **70**: 4748–4755.

Steward GF, Jenkins BD, Ward BB, Zehr JP. 2004. Development and testing of a DNA macroarray to assess (*nifH*) gene diversity. *Applied and Environmental Microbiology* **70**: 1455–1465.

Stralis-Pavese N, Sessitsch A, Weilharter A, Reichenauer T, Riesing J, Csontos J, Murrell JC, Bodrossy L. 2004. Optimisation of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers. *Environmental Microbiology* **6**: 347–363.

Svenning MM, Wartiainen I, Hestnes AG, Binnerup SJ. 2003. Isolation of methane oxidising bacteria from soil by use of a soil substrate membrane system. *FEMS Microbiology Ecology* **44**: 347–354.

Taroncher-Oldenburg G, Griner EM, Francis CA, Ward BB. 2003. Oligonucleotide microarray for the study of functional gene diversity in the nitrogen cycle in the environment. *Applied and Environmental Microbiology* **69**: 1159–1171.

Tiquia SM, Wu L, Chong SC, Passovets S, Xu D, Xu Y, Zhou J. 2004. Evaluation of 50-mer oligonucleotide arrays for detecting microbial populations in environmental samples. *Bio/Techniques* **36**: 664–665.

Torsvik V, Øvreås L. 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion Microbiology* **5**: 240–245.

Torsvik V, Goksøyr J, Daae FL. 1990. High diversity of DNA of soil bacteria. *Applied and Environmental Microbiology* **56**: 782–787.

Torsvik V, Sørheim R, Goksøyr J. 1996. Total bacterial diversity in soil and sediment communities – a review. *Journal of Indian Microbiology* **17**: 170–178.

Urakawa H, El Fantroussi S, Smidt H, Smoot JC, Tribou EH, Kelly JJ, Noble PA, Stahl DA. 2003. Optimization of single-base-pair mismatch discrimination in oligonucleotide microarrays. *Applied and Environmental Microbiology* **69**: 2848–2856.

Volokhov D, Pomerantsev A, Kivovich V, Rasooly A, Chizhikov V. 2004. Identification of *Bacillus anthracis* by multiprobe microarray hybridization. *Diagnosis of Microbiological Infectious Diseases* **49**: 163–171.

Voordouw G, Voordouw JK, Karkhoff-Schweizer RR, Fedorak PM, Westlake DWS. 1991. Reverse sample genome probing, a new technique for identification of bacteria in environmental samples by DNA hybridization and its application to the identification of sulfate-reducing bacteria in oil field samples. *Applied and Environmental Microbiology* **57**: 3070–3078.

Vora GJ, Meador CE, Stenger DA, Andreadis JD. 2004. Nucleic acid amplification strategies for DNA microarray-based pathogen detection. *Applied and Environmental Microbiology* **70**: 3047–3054.

Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, Derisi JL. 2002. Microarray-based detection and genotyping of viral pathogens. *Proceedings of the National Academy of Sciences, USA* **99**: 15687–15692.

Warsen AE, Krug MJ, LaFrentz S, Stanek DR, Loge FJ, Call DR. 2004. Simultaneous discrimination between 15 fish pathogens by using 16S ribosomal DNA PCR and DNA microarrays. *Applied and Environmental Microbiology* **70**: 4216–4221.

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A. 2004. Diversity arrays technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Sciences, USA* **101**: 9920.

Wertz JE, Goldstone C, Gordon DM, Riley MA. 2003. A molecular phylogeny of enteric bacteria and implications for a bacterial species concept. *Journal of Evolutionary Biology* **16**: 1236–1248.

Wetmur JG. 1991. DNA probes: applications of the principles of nucleic acid hybridization. *Critical Reviews in Biochemistry and Molecular Biology* **26**: 227–259.

Wilson KH, Wilson WJ, Radosevich JL, DeSantis TZ, Viswanathan VS, Kuczmarski TA, Andersen GL. 2002. High-density microarray of small-subunit ribosomal DNA probes. *Applied and Environmental Microbiology* **68**: 2535–2541.

Wind T, Stubner S, Conrad R. 1999. Sulfate-reducing bacteria in rice field soil and on rice roots. *Systematic and Applied Microbiology* **22**: 269–279.

Wittenberg AHJ, van der Lee T, Cayla C, Kilian A, Visser RGF, Schouten HJ. 2005. Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana. Molecular Genetics and Genomics* **274**: 30–39.

Wodicka L, Dong HL, Mittmann M, Ho MH, Lockhart DJ. 1997. Genome-wide expression monitoring in *Saccharomyces cerevisiae. Nature Biotechnology* **15**: 1359–1367.

Wong RSY, Chow AW. 2002. Identification of enteric pathogens by heat shock protein 60 kDa (HSP60) gene sequences. *FEMS Microbiology Letters* **206**: 107–113.

Wu L, Thompson DK, Li R, Hurt RA, Tiedje JM, Zhou J. 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. *Applied and Environmental Microbiology* **67**: 5780–5790.

Wu L, Thompson DK, Liu X, Fields MW, Bagwell CE, Tiedje JM, Zhou J. 2004. Development and evaluation of microarray-based whole-genome hybridization for detection of microorganisms within the context of environmental applications. *Environmental Science and Technology* **38**: 6775–6782.

Xia L, Peng K, Yang S, Wenzl P, de Vicente C, Fregene M, Kilian A. 2005. DArT for high-throughput genotyping of cassava (*Manihot esculenta*) and its wild relatives. *Theoretical and Applied Genetics* **110**: 1092–1098.

Ye RW, Tao W, Bedzyk L, Young T, Chen M, Li L. 2000. Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions. *Journal of Bacteriology* **182**: 4458–4465.

Zhou J. 2003. Microarrays for bacterial detection and microbial community analysis. *Current Opinion Microbiology* **6**: 288–294.

Zwieb C, Gorodkin J, Knudsen B, Burks J, Wower J. 2003. tmRDB (tmRNA database). *Nucleic Acids Research* **31**: 446–447.

About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at **www.newphytologist.org**.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – the 2004 average submission to decision time was just 30 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £109 in Europe/$202 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (**newphytol@lancaster.ac.uk**; tel +44 1524 594691) or, for a local contact in North America, the US Office (**newphytol@ornl.gov**; tel +1 865 576 5261).

