Advantages and disadvantages of microarrays to study microbial population dynamics – a minireview

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Abstract Microbial ecology is challenging because of practical problems associated with detecting and quantifying populations. Bacteria and yeasts are not easily identified using conventional methods such as dilution plating and biochemical tests. Those methods lack sensitivity, are extremely time-consuming and cannot account for unculturable organisms. New DNA-based technology, such as microarrays, offers solutions to those problems. However, identification of microorganisms using DNA methodology is becoming increasingly complicated due to the variation revealed in sequenced microbe genomes. Identification is no longer considered reliable when only one area of the genome is targeted, and recent publications consider that sequences from six or seven different genes are required to resolve species or pathovars of fungi and bacteria reliably. A large number of probes from different genes can be included on a single microarray chip. The advantages and disadvantages of microarrays versus other DNA methods for studying microbial ecology of fruit surfaces are discussed.

Keywords microbial ecology, quantitative PCR, bacteria, fungi, fructosphere, DNA.

INTRODUCTION

Population dynamics of microbial communities can be studied using various techniques including dilution plating, microscopy, denaturing gel gradient electrophoresis (DGGE), quantitative polymerase chain reaction (qPCR), and microarrays. Each method has advantages and disadvantages that have been previously reviewed (Everett et al. 2005). Since that review, microarrays have been increasingly used in studies of microbial ecology, and next generation sequencing has become available. These new applications and examples are reviewed here.

DEVELOPMENT OF ARRAYS

Microarrays were first developed as a modification of dot blotting, which is an application of the standard molecular technique, Southern blotting (Southern 1975). Dot blotting consisted of placing a small amount of fragmented genomic DNA (gDNA, the target) onto a nitrocellulose or nylon membrane, then hybridising with a radioactively labelled DNA probe that would only bind to a known region of DNA diagnostic for identification. The radioactive signal was able to be detected using X-ray film. Macroarrays were developed to increase the number of different

DNA probes that could be assessed concurrently and, in contrast to dot-blotting, the probes not the target were placed on the membrane using a pin-spotting device. The number of spots (probes) that are typically able to be placed on a macroarray are about 50 at 250 µm spacing. This enabled a labelled target to be screened against many probes, each specific for a different gene region. Later miniaturised microarrays were developed that allowed a far larger number of DNA probes to be placed on silicon or glass substrates (Schena et al. 1995), and fluorescent dyes were used to detect successful hybridisation. Typically 50,400 spots 70 µm apart can be placed on one glass microarray slide. Microarrays were first used for gene expression profiling. Later applications of microarray technology included comparative genomic hybridisation (Pollack et al. 1999) and single nucleotide polymorphism (SNP) detection (Hacia et al. 1999).

ARRAYS VERSUS REAL-TIME PCR

The real-time, or quantitative, polymerase chain reaction (qPCR) technique offers rapid field diagnosis of plant disease, especially following the development of low-cost portable qPCR machines developed for rapid assessment of bioterrorism threats by the USA army (Vincelli & Tisserat 2008). Quantitative PCR is able to estimate the quantity of DNA that is in a sample, and examination of the melting curves can confirm specificity. Oligonucleotide arrays offer a far greater capacity for multiplexing than qPCR. Arrays have the advantage of allowing researchers to concurrently screen for a far greater number of potential pathogens than qPCR techniques. As an example, Tambong et al. (2006) developed an array that can identify virtually all 100 known species of *Pythium*. Use of this overnight array technique allows the researcher to identify unknown *Pythium* strains to species level rather than using 100 species-specific primers in qPCR or PCR reactions over at least 2 days. A further advantage of the multiplexing capability of microarrays for pathogen identification is that several probes for each taxon can be designed to different gene regions, for more robust

identification aligned with recent advances in phylogenetics (Sarkar & Guttman 2004).

However, qPCR is potentially more sensitive than microarrays, can quantify the amount of DNA, and can detect as little as 0.1 fg of gDNA, as was demonstrated for *Phytophthora fragariae* (O'Brien et al. 2009). In comparison a detection limit of 1 fg of DNA for microarray detection has been demonstrated for *Escherichia coli* (Call et al. 2001). This problem can be overcome by increasing the sample size and concentrating the genetic material by centrifugation.

MICROARRAYS VERSUS MACROARRAYS

Macroarrays require less specialised equipment than microarrays and can be used in any reasonably well set up molecular laboratory. However, macroarrays have only a moderate throughput and the binding of oligonucleotides is not controlled (Anthony et al. 2000; Rudi et al. 2002). Other workers point out that macroarrays can be more sensitive than microarrays because spots on a macroarray contain higher amounts of detector oligonucleotides (Cho & Tiedje 2002). When using microarrays these workers could not detect 10⁵ cells of *Pseudomonas stutzeri* in soil, which equates to 50 ng of *P. stutzeri* genome. In contrast, they comment that macroarray technology can detect femtogram quantities. However, later workers clearly demonstrated that microarray sensitivity can be improved by using high copy number target DNA regions (such as rRNA) and a PCR labelling step that amplifies the query region of the organism of interest. As little as 100 fg of *E. coli* genomic DNA was detected using these improvements (Lee et al. 2006) and a detection limit of 1 fg for microarrays has also been reported (Call et al. 2001). However, this level of sensitivity is limited to one gene region with high copy number (rDNA).

MICROARRAY DEVELOPMENT- WHY SO SLOW?

Microarrays have been developed as diagnostic tools for identifying bacterial pathogens in food (Rasooly & Herold 2008), diagnosis of human fungal pathogens (Lau et al. 2009), identification

of new strains of human viruses, including SARS and the H1N1 strain of the influenza virus (Townsend et al. 2006; Kang et al. 2010), and for the identification of European quarantine plant pathogenic bacteria (Pelludat et al. 2009). In a recent review Bodrossy & Sessitsch (2004) commented that the transition from technology development to application is relatively slow for microarrays. Publications in the area were most commonly of technology development rather than testing potential commercial applications. In our experience the validation and design of specific oligonucleotides for robust identification of biosecurity risk pathogens can be extremely time-consuming due to misidentification of organisms deposited in culture collections (Rees-George et al. 2010), and revision of phylogenies due to genetic analysis of taxonomically informative gene regions using molecular techniques (Sarkar & Guttman 2004). Indeed, Frey et al. (2009) comment that arrays have lagged far behind their potential because of the paucity of discriminatory gene regions available for species identification. Another problem in the microbial diagnostic community is the lack of consensus of what gene regions should be used for identification and classification. This lack of consensus over discriminatory regions for identification is a limiting step for all diagnostic molecular methods, including next-generation sequencing.

MICROARRAYS VERSUS SEQUENCING

Sequencing will detect unknown organisms whereas arrays will only detect known organisms. However, if the aim of the study is to detect known organisms, then sequencing can generate a large number of sequences that are not of interest because either they are not informative for identification, or they contain sequences from other organisms e.g. saprotrophs, symbionts or secondary colonisers. Consequently the bioinformatics processing task, essential for detection by sequencing, is unnecessarily large and time-consuming.

Although microarrays are not as good as sequencing for detecting unknown microbes, microarray technology allows greater replication

(both of probes and slides) and reveals greater diversity in environmental samples (DeSantis et al. 2007). This is because of the limited number of clones that were used; fewer than 1000 sequences using a cloned library of 16S rRNA gene fragments. In contrast, the high density microarray consisted of 297,851 probes. For soil samples, the array could discriminate 194 different Family groups, but the cloned library could discriminate only three Family groups. Next-generation sequencing techniques do not require a clone library, and thus discrimination should improve, but recent reviewers comment that although next-generation sequencing is becoming more accessible to researchers, microarrays will probably still be used for microbial ecology studies. Microarrays “appear to be the ideal tool to assess the diversity of the microbial world” (Huyghe et al. 2009). However, further development of next-generation sequencing technologies may increase their suitability for studying environmental samples.

USING MICROARRAYS TO STUDY MICROBIAL POPULATIONS

Macro- and microarrays have been used for studying populations of microorganisms in clinical samples (Wang et al. 2002a), the human intestine (Wang et al. 2002b), human sputum (Fukushima et al. 2003), air filtrates (Wilson et al. 2002), soil, compost or sludge (Stralis-Pavese et al. 2004; Lievens et al. 2005; Kyselkova et al. 2009b), ectomycorrhizal fungal communities in soil (Reich et al. 2009), human pathogens or other bacteria in water (Peplies et al. 2004; Urakawa et al. 2007), and human pathogens in milk (Giannino et al. 2009). Using microarrays to study microbial populations on plant surfaces is less common, and only one example of a study of microbial populations on ready-to-eat vegetable salads could be found (Rudi et al. 2002).

Microarray applications for studying plant pathogens have so far consisted of monitoring differences in soil pathogen populations. For example, bacterial populations of soils that are suppressive or conducive to black root rot disease (*Thielaviopsis basicola*). Kyselkova et al. (2009a)

designed a microarray that contained 1033 probes targeting 19 bacterial phyla including Proteobacteria, Firmicutes, Actinomycetes, Cyanobacteria and Bacteroidetes. Results clearly showed that the microarray data discriminated between black root rot-suppressive and -conductive soils. In addition, Lievens et al. (2005) developed a macroarray that was able to quantify the vascular wilt pathogens *Verticillium albo-atrum* and *V. dahliae* in soil, but follow-up studies using this technique have not been published. Elsewhere there is discussion on the complications of base pair mismatches and of the probe dependency of signal intensity that impact on quantification. A solution to both these complications is the use of padlock probes that only ligate to perfectly matched targets (Kyselkova et al. 2009b). Absolute quantification is not possible with this technique, although it is possible to determine differences between samples, but not between individual microorganisms in one sample.

COMPARATIVE COSTS OF MICROARRAYS

Microarrays have been criticised as expensive for routine use. Historically molecular equipment costs have reduced over time as sales volume increases. For example qPCR machines are now 10% of the cost when they were first developed 10 years ago. Cheaper microarray systems such as Clontech (<http://www.biochipnet.com/node/154>), which are currently being commercialised, use a distinct reader system that reduces costs (Barrett et al. 2009). Comparisons of the timeliness and accuracy of these microarray methods with conventional culturing and biochemical tests for bacterial identification have concluded that the microarrays are superior and less labour intensive. qPCR has high costs for consumables, and robust identification of many different organisms can require several runs, thus increasing the labour component costs. The costs of next-generation sequencing are reducing as the technology improves, but are still prohibitive for routine diagnostics that do not include multiplexing through the use of identifying 'barcodes' at the end of primers. For all of these molecular diagnostic methods,

the greatest cost is in the identification and testing of discriminatory gene regions that can be used for identification of the organism. A large international cooperative effort is required to generate diagnostic oligonucleotides. Once these become public domain the costs will reduce further.

CONCLUSIONS

Microarrays are the method of choice for studying diversity of microbial communities, and will continue to be used despite the advent of accessible next-generation sequencing techniques. Relative quantification of differences between samples is possible using this technique. For diagnostics, microarrays still offer a number of advantages over other techniques because of the high capacity for multiplexing. The step that is limiting the commercialisation and further development of microarrays for organism identification is the complexity and the time required to design and test discriminatory genetic regions that separate one species from another. This same lack of discriminatory information also limits other molecular identification methods, including sequencing.

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REFERENCES

- 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.
- Barrett B, Adams I, Glover R, Boonham N 2009. The development of a Clontech potato virus microarray for diagnostic use. *Aspects of Applied Biology* 94: 49-50.
- Bodrossy L, Sessitsch A 2004. Oligonucleotide microarrays in microbial diagnostics. *Current Opinion in Microbiology* 7: 245-254.

- Call DR, Brockman FJ, Chandler DP 2001. Detecting and genotyping *Escherichia coli* O157:H7 using multiplexed PCR and nucleic acid microarrays. *International Journal of Food Microbiology* 67 (1-2): 71-80.
- Cho J-C, Tiedje JM 2002. Quantitative detection of microbial genes by using DNA microarrays. *Applied and Environmental Microbiology* 68: 1425-1430.
- DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL 2007. High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. *Microbial Ecology* 53 (3): 371-383.
- Everett KR, Vanneste JL, Hallett IC, Walter M 2005. Ecological alternatives for disease management of fruit rot pathogens. *New Zealand Plant Protection* 58: 55-61.
- Frey JE, Pasquer F, Pelludat C, Duffy B 2009. A high-density random-oligonucleotide genome microarray for universal diagnostics. *Bulletin OEPP/EPPO Bulletin* 40 (1): 40-45.
- Fukushima M, Kakinuma K, Hayashi H, Nagai H, Ito K, Kawaguchi R 2003. Detection and identification of *Mycobacterium* species isolates by DNA microarray. *Journal of Clinical Microbiology* 40: 2605-2615.
- Giannino ML, Aliprandi M, Feligini M, Vanoni L, Brasca M, Fracchetti F 2009. A DNA array based assay for the characterization of microbial community in raw milk. *Journal of Microbiological Methods* 78 (2): 181-188.
- Hacia JG, Fan JB, Ryder O, Jin L, Edgemon K, Ghandour G, Mayer RA, Sun B, Hsie L, Robbins CM, Brody LC, Wang D, Lander ES, Lipshutz R, Fodor SP, Collins FS 1999. Determination of ancestral alleles for human single-nucleotide polymorphisms using high-density oligonucleotide arrays. *Nature Genetics* 22: 164-167.
- Huyghe A, Francois P, Schrenzel J 2009. Characterization of microbial pathogens by DNA microarrays. *Infection, Genetics and Evolution* 9 (5): 987-995.
- Kang X, Li Y, Sun H, Wu W, Liu H, Lin F, Qing C, Chang G, Zhu Q, Chen W, Yang Y 2010. Subtype identification of the novel A H1N1 and other human influenza A viruses using an oligonucleotide microarray. *Archives of Virology* 155 (1): 55-61.
- Kyselkova M, Kopecky J, Frapolli M, Defago G, Sagova-Mareckova M, Grundmann GL, Moenne-Loccoz Y 2009a. Comparison of rhizobacterial community composition in soil suppressive or conducive to tobacco black root rot disease. *ISME Journal* 3 (10): 1127-1138.
- Kyselkova M, Kopecky J, Sagova-Mareckova M, Grundmann GL, Moenne-Loccoz Y 2009b. Oligonucleotide microarray methodology for taxonomic and functional monitoring of microbial community composition. *Plant, Soil and Environment* 55 (9): 379-388.
- Lau A, Chen S, Sleiman S, Sorrell T 2009. Current status and future perspectives on molecular and serological methods in diagnostic mycology. *Future Microbiology* 4 (9): 1185-1222.
- Lee DY, Shannon K, Beaudette LA 2006. Detection of bacterial pathogens in municipal wastewater using an oligonucleotide microarray and real-time quantitative PCR. *Journal of Microbiological Methods* 65 (3): 453-467.
- Lievens B, Brouwer M, Alfons CRC, Vanachter ACRC, Levesque CA, Cammue BPA, Thomma BPHJ 2005. Quantitative assessment of phytopathogenic fungi in various substrates using a DNA macroarray. *Environmental Microbiology* 7 (11): 1698-1710.
- O'Brien PA, Williams N, Hardy GE 2009. Detecting *Phytophthora*. *Critical Reviews in Microbiology* 35 (3): 169-181.
- Pelludat C, Duffy B, Frey JE 2009. Design and development of a DNA microarray for rapid identification of multiple European quarantine phytopathogenic bacteria. *European Journal of Plant Pathology* 125 (3): 413-423.
- Peplies J, Lau SCK, 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.

- Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO 1999. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nature Genetics* 23 (1): 41-46.
- Rasooly A, Herold KE 2008. Food microbial pathogen detection and analysis using DNA microarray technologies. (Special issue on the application of "omics" technologies for food safety research.). *Foodborne Pathogens and Disease* 5 (4): 531-550.
- Rees-George J, Vanneste JL, Cornish DA, Pushparajah IPS, Yu J, Templeton MD, Everett KR 2010. Detection of *Pseudomonas syringae* pv. *actinidiae* using polymerase chain reaction (PCR) primers based on the 16S-23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions. *Plant Pathology* 59: 453-464.
- Reich M, Kohler A, Martin F, Buee M 2009. Development and validation of an oligonucleotide microarray to characterise ectomycorrhizal fungal communities. *BMC Microbiology* 9: 241.
- Rudi K, Flateland SL, Hanssen JF, Bengtsson G, Nissen H 2002. Development and evaluation of a 16S ribosomal DNA array-based approach for describing complex microbial communities in ready-to-eat vegetable salads packed in a modified atmosphere. *Applied and Environmental Microbiology* 68: 1146-1156.
- Sarkar SF, Guttman DS 2004. Evolution of the core genome of *Pseudomonas syringae*, a highly clonal, endemic plant pathogen. *Applied and Environmental Microbiology* 70 (4): 1999-2012.
- Schena M, Shalon D, Davis RW, Brown PO 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science of the Total Environment* 270 (5235): 467-470.
- Southern EM 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98: 503-517.
- Stralis-Pavese N, Sessitsch A, Weilharter A, Reichenauer TG, 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.
- Tambong JT, de Cock AWAM, Tinker NA, Levesque CA 2006. Oligonucleotide array for identification and detection of *Pythium* species. *Applied and Environmental Microbiology* 72 (4): 2691-2706.
- Townsend MB, Dawson ED, Mehlmann M, Smagala JA, Dankbar DM, Moore CL, Smith CB, Cox NJ, Kuchta RD, Rowlen KL 2006. Experimental evaluation of the FluChip diagnostic microarray for influenza virus surveillance. *Journal of Clinical Microbiology* 44 (8): 2863-2871.
- Urakawa H, Matsumoto J, Hoshino T, Tsuneda S 2007. Direct profiling of rRNA in saline wastewater treatment samples using an oligonucleotide microarray. *Microbes and Environments* 22 (2): 116-122.
- Vincelli P, Tisserat N 2008. Nucleic acid-based pathogen detection in applied plant pathology. *Plant Disease* 92 (5): 660-669.
- Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, Derisi JL 2002a. Microarray-based detection and genotyping of viral pathogens. *Proceedings of the National Academy of Sciences USA* 99: 15687-15692.
- Wang RF, Beggs ML, Robertson LH, Cerniglia CE 2002b. Design and evaluation of oligonucleotide-microarray method for the detection of human intestinal bacteria in fecal samples. *FEMS Microbiology Letters* 213: 175-182.
- 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.