



DNA arrays applied to microorganisms detection

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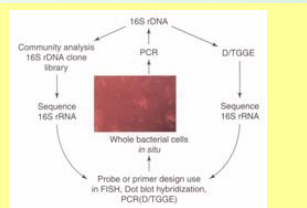
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Material and Methods

A DNA array for the identification of Gram+ and Gram- bacteria based on 16S rDNA and other genes with specificity at genus and species level has been developed. The chip was made with 30 oligonucleotide (20mer) sequences (11 from 16S rDNA). PCR amplification products were used as probes. PCR reactions using an excess of reverse primer in asymmetric PCR amplification produced single strand DNA performing fast hybridisations. DNA extraction steps from various matrices were optimized on the microorganisms to be detected.

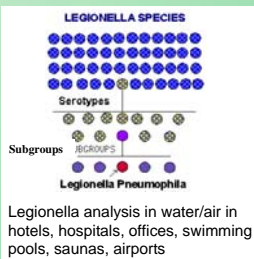
18-21mer oligonucleotides were designed and spotted onto slides at a concentration 50µM (3X SSC). 11 probes were designed on 16S, and other single copy specie-specific genes as *GroEL/Hsp60* chaperonin and *tuf*. To test the efficacy of the method 16SrDNA probes for *E.coli 1105*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium difficile*, were spotted in first instance together with LacZ probe for *E. coli*, probes for *stx1* and *stx2* for *E. coli*, *nuc* probe for *Staphylococcus aureus*, *emo/ahs* and *cutC* genes for *Listeria monocytogenes*, and a *mip* probe for *Legionella pneumophila*. Primers to amplify the region of interest were designed. Cultures of species of interest were grown in liquid medium and DNA was extracted and purified. Nexterion slides E (epoxy-slides) were used according to manufacturer instruction (Schott, Germany). Briefly, after initial washing slides were blocked in blocking buffer (ethanolamine 50mM, 0,01% SDS, 0,1MTris-HCl pH9) at 50°C for 15 min.

DNA products were Cy3-labeled during the PCR amplification reaction and purified by gel filtration. Fluorescent dye incorporated in 1µl of PCR products were done using a Nanodrop spectrofluorimeter. Intensity values of cyanine were equal or higher than **0,6 pmol/µl for 100 ng** of PCR product. The labelled target was added to hybridization buffer (3X SSC, 50% formamide, 0,2% SDS). After 3 min denaturation at 95°C, DNA target was hybridized on slides at 42°C overnight. Washing was performed in serial dilutions of SSC. Signal analysis made on Affymetrix 428 scanner.

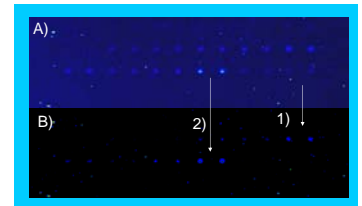


Culture-dependent and culture-independent methods for analysis of bacteria and species identification.

S. aureus analysis methods with species-specific genes: 1) staphylococcal enterotoxins (SE) typing at sub-species level, 2) *nuc* and *femA* gene sequences, 3) *met* sequence for methicillin resistance (MRSA), 4) Detection of multidrug and toxin extrusion (MATE) MDR efflux proteins and *MepA* genes.



Legionella analysis in water/air in hotels, hospitals, offices, swimming pools, saunas, airports



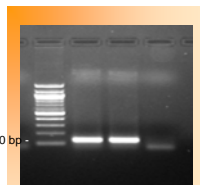
E- coli hybridisation results, A: scheme of 11 16S rDNA based oligos, spotted in duplicate. B: 1) specific binding of the E. coli probe to E. coli oligonucleotides; 2) positive control. Several probes were changed due to aspecific binding.

Different labeling methods were compared: direct and indirect labeling, asymmetric PCR.

CONCLUSIONS

The DNA array protocol took 24 hours for performing all the steps: it showed to be a fast multiplex analysis. DNA arrays set up has the capacity to verify the presence of many species in a single run. This characteristic overcomes a relatively long time to obtain results when compared to other molecular methods such as real time PCR. Ongoing work is aimed at designing new oligonucleotides based on single copy genes such as *rpoB*, *tuf* and *GroEL/Hsp60* chaperone allowing higher species-specific resolution.

Detection of bacteria from real samples (Food, meat, water, blood)
Improvements on traditional protocols: Extraction of DNA
Bacteria colonizing surfaces, such as *Listeria* species, were harvested from samples by repeated washings. Proprietary buffer was used to eliminate blood cells and hemoglobins. Additional enrichment step based on filtration was performed. Bacteria were lysed in the presence of lysozyme, using proteinase K when necessary, then DNA was purified on silica or magnetized particles. PCR amplification products were obtained in 100% of cases.



Control of *Listeria ahs* PCR products by agarose gel electrophoresis

Comparison with traditional PCR method in species identification

PRODUCTIVITY	qPCR, RT-PCR/DNA sequencing Dot hybridisation	DNA array System
Productive outcome	Multiplexing	Many complex, many genes/sample
Detection	molecular bacteria, 4 color-lasers	Quantify the abundance, microfluidics on-chip assembling and processing
Maintenance rate	High (with many use automated steps, manually conducted by operators)	Facility for automation, microfluidics on-chip assembling and processing
Maintenance time	From hours to 48 hours	From hours to 24 hours
Sensitivity	From 100 to 1000 cells/CFU	From 10 to 100 cells

Applicability	Example(s)
Food Industry	Fish, meat or food products
Bioprocess Monitoring	Microorganisms monitoring and control
Healthcare Industry	Speedy recognition of bacteria used against hazardous bacteria in blood, other organs, legals or pathogens control tests
Environmental Monitoring	Hazardous waste monitoring
Agriculture & Related Industries	Analysis of plant parasite
Research & Development	More rapid gene sequencing