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DNA-based methods for detection of food-borne bacterial pathogens

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Abstract

Food-borne diseases are mainly caused by pathogenic bacteria, which are transmitted to humans from the animal reservoir via food or which contaminates the food on the processing line. Detection of the pathogenic bacteria constitutes a challenge, as the bacteria are often present in low numbers, masked by the food matrix and outnumbered by high numbers of indigenous bacteria. Traditional detection methods, therefore, include enrichment and often pre-enrichment steps, which are time and labour consuming. Many different techniques have been developed to speed up detection of pathogenic bacteria and to increase the sensitivity of the detection. In the current paper, the state of the art for DNA-based detection methods are reviewed with a view to their limitations in relation to food microbiology.

Keywords: DNA-probes; PCR; Oligonucleotide arrays; Bacterial pathogens

1. Introduction

Food-borne diseases are mainly caused by pathogenic bacteria which are either transmitted to humans from the animal reservoir or which contaminate the food process line. Detection and isolation of these bacteria from food are often difficult due to the high number of contaminating and indigenous bacteria and a low number of the pathogenic bacteria of concern. In order to obtain even a modest sensitivity, most traditional isolation methods include a selective enrichment and sometimes a pre-enrichment step, both of which are labour and time consuming. There is, consequently, scope for improvement of detection and isolation methods, especially with respect to the time needed to produce a diagnosis.

The last 20–30 years have seen many developments in techniques and also the dawning of technologies, which were predicted to change our ways of detecting pathogenic bacteria in food. Table 1 lists examples of technologies which have been applied to improve methods for detection of pathogenic bacteria in food. Some of the techniques were first targeted towards use in clinical microbiology, where they have obtained a wide use; colony hybridization (Grundstein & Hogness, 1975), polymerase chain reaction (PCR) (Saiki et al., 1988), and different antibody based techniques, such as immunomagnetic separation (IMS) (Olsvik et al., 1994), are examples of such methods.

Several reviews have dealt with the use of DNA-probes and the PCR-technique in food microbiology (Hill, 1996; Hill & Keasler, 1991; Olsen, Aabo, Hill et al., 1995; Wolcott, 1991). Based on the wide use that the techniques quickly were put to for research purposes, the reviews unanimously praise the potential of these techniques to overcome some of the inherent problems in detection and isolation of bacterial pathogens from food. Seen in the mirror, the DNA-technologies, like most other new technologies, have only found use for very specific purposes, while the vast majority of food analyses are still carried out by the use of the well known culture techniques. This review will summarise the state of art for DNA-based methods for detection and isolation of food-borne bacterial pathogens, as an illustration of, which obstacles any new method has to overcome to be suitable for routine analysis. The description will focus on the applications, where DNA-based methods can be an advantage to routine food microbiology. With a view to the future, oligonucleotide arrays, which are predicted to become the method of choice for species-specific identification of bacteria, will also briefly be discussed in relation to the use in food microbiology.

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Table 1
Examples of new methods for detection and/or isolation of food-borne bacterial pathogens

Principle	Example of methodology
Improved (automatic) viable count	Spiral plate count Hydrophobic grid-membrane filter technique
Microscopy techniques	Direct epifluorescence filter technique (DEFT) Flow cytometry Fluorescence antibody technique
Electric changes	Impedence methods
Estimation of metabolic activity	ATP assays
Antibody/antigen reactioin	Latex agglutination Immunomagnetic separation Enzyme linked immunosorbent assays (ELISA)
DNA-detection	Colony hybridization Single phase hybridization assays Polymerase chain reaction (PCR) Oligonucleotide arrays (DNA-disc technology)

2. The methodology of DNA-based methods for detection and identification of pathogenic bacteria

The DNA-based detection methods have now been around for the best part of 20 years, and the methodologies are well known to most people dealing with food microbiology. A brief description will be given below, while the reader is referred to more detailed works for protocols and a full-covering of applications (Hill, 1996; Hill & Keasler, 1991; Wolcott, 1991). Table 2 summarizes the general advantages and disadvantages of DNA-based methods in relation to food microbiology.

The ability of two single stranded DNA-molecules in vitro, under the right conditions, to form double stranded DNA by specific base pairing, i.e to hybridise, is the basis of all DNA-based detection methods. While many different methods based on specific base pairing have been developed (Wolcott, 1992), three methods, i.e. colony hybridization (Grundstein & Hogness, 1975), single phase, liquid hybridization assays (Curiale, Klatt & Mozola, 1990a), and PCR (Saiki et al., 1988) are in particular relevant for detection of bacteria in food.

In the colony hybridization assay, a food sample or an enrichment culture is spread on a nylon or paper filter and incubated until visible colonies are present. These are processed to destroy the cells, remove cell substances and leave fixed single-stranded DNA for hybridisation, usually by treatment with detergent and alkali or by microwave treatment as in the method of Datta, Wentz and Hill (1987). A radio, enzyme or hapten labelled DNA-probe, which constitutes part of the target DNA-sequence, is applied to hybridise to the sample DNA. Each signal on the filter corresponds to a positive identification (Fig. 1), and upon direct spreading of samples, colony hybridisation is a quantitative method. Since oligonucleotides can be synthesised in vivo, short, defined and synthetic oligonucleotides were quickly introduces into food microbiology (Hill, Payne, Zon & Moseley, 1985) and are now by far preferred as probe molecules.

An essential step in colony hybridisation is the separation between labelled probe molecules bound to the target DNA and those that bind non-specifically to the filter. This is obtained by stringency washing, which is only possible when the target DNA is fixed to a solid support, i.e. the filter. This in terms, however, cost a considerable decrease in the speed of the hybridisation, possibly because of stearic hindrance (Meinkoth & Wahl, 1984). In an attempt to overcome this problem, assays have been developed, where the hybridisation between target DNA and probe takes place in solution (one phase hybridisation assays). The hybrids formed

Table 2
A summary of general advantages and problems of DNA-based methods for detection and characterisation of food-borne bacterial pathogens compared to traditional culture based methods

Application	Advantages	Problems
Direct detection of pathogens in food.	Does not (in theory) require cultivation.	May detect dead cells (PCR). High detection level due to small test volume. Inhibition due to food components (PCR). Very sensitive to laboratory contamination (PCR).
Culture confirmation on enrichment broths	Rapid and sensitive. Automatic detection possible. Insensitive to high concentrations of indigenous microbiota.	No colony obtained, and hence most suitable for screening out negative samples.
Characterization of pathogenic bacteria obtained from food samples.	Can separate virulent from non-virulent bacteria by targeting virulence genes. Multiplex characterization possible (PCR).	
Other applications	Can specifically detect genetically modified organisms (GMOs).	

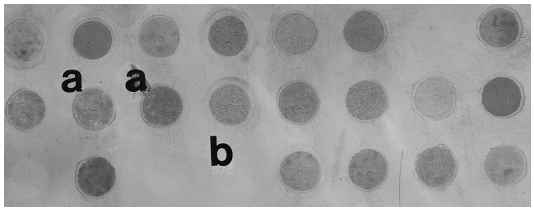

Fig. 1. DNA-colony hybridisation. The result of a colony hybridisation is demonstrated. Forty-four broths were spotted onto a filter and hybridised with a *Salmonella* specific DNA-probe. a: positive spots.

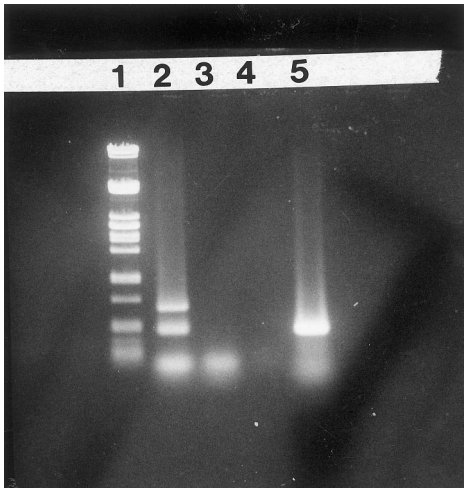

Fig. 2. The polymerase chain reaction (PCR). The result of a PCR reaction is shown in the form of an agarose gel with amplified DNA-fragments. DNA was obtained from broth cultures and amplified with two sets of primers (multiplex PCR). One set of primers amplifies a DNA fragment in members of the genus *Salmonella* and one set of primers amplifies a fragment in members of the serotype *S. typhimurium*. Lane 1 is a molecular size marker, lane two shows the result from a strain of *S. typhimurium* (two bands), lanes three and four are negative control and a negative sample and lane 5 is a strain of *S. dublin* with the genus specific band only. Faint bands at the bottom are primer-dimers.

are captured afterwards and signals are developed These assays are all targeted towards the variable regions of the ribosomal RNA (rRNA), and the high concentration of rRNA in the ribosomes of the growing bacteria then becomes part of the target, which greatly increases the speed of the reaction.

In the PCR method, a specific amplification of a defined target-DNA is obtained by successive cycles of three steps, namely denaturation of sample DNA to obtain single strand target, annealing of short and specific primers to the target DNA and polymerisation of DNA starting from the primers by use of a thermostable DNA-polymerase, derived from the thermophilic aquatic bacteria, *Thermus aquaticus* (Saiki et al., 1988). The number of amplicons are doubled in each cycle, and all but the fragments produced with the original target DNA as template will be of a fixed size, corresponding to the distance between the two primers. Detection of the positive reaction can be done simply by demonstrating the correct DNA-fragment by agarose-gel electrophoresis (Fig. 2). However, in general the specificity should be controlled by hybridisation using an internal DNA probe, digestion with a restriction enzyme or DNA sequencing of the amplicon, as some oligonucleotides prime the formation of DNA-fragments of several sizes and base compositions. For use in routine laboratories, systems in which the signal is quantitative and hence can be compared to a cut off value, have been developed (Holmstrøm, Rossen & Rasmussen, 1993; Rasmussen, Christensen & Olsen, 1995; Uhlén, 1990). Another way of increasing the specificity of PCR is to combine two PCR-methods (nested PCR). In this method, a second set of primers is used to amplify a region within the first PCR-product. In this way, a signal will be produced if the correct fragment was amplified in the first round, while "false amplicons' are ignored. Semi-automated PCR-machines are now on the market, making a quantitative determination of pathogenic bacteria from pure cultures possible.

3. The use of colony-hybridization in food microbiology

Moseley, Huq, Alim, So, Samadpour-Motalebi and Falkow (1980) introduced colony hybridisation to diagnostic microbiology when they used radio-labelled cloned fragments of the heat labile and heat stable enterotoxin genes from *Eschichera coli* to detect ETEC among pure cultures of bacteria and in human stools spotted directly on nitrocellulose filters. Hill (1981) modified this method, and used it to enumerate ETEC in the presence of more than 10^6 CFU of contaminating bacteria. He also illustrated the potential for application to food microbiology by detecting ETEC by colony hybridisation in spiked scallop without prior enrichment. Since then, specific DNA probes in the form of both polynucleotides and oligonucleotides have been developed for all the important food-borne bacterial pathogens (Table 3).

The method is relatively insensitive to high background microbiota when radio-labelled probes are used (Datta, Wentz & Hill, 1988; Jagow & Hill, 1986). Even when problems do arise, i.e. when the total count reaches around 10^7 CFU/gram, this can relatively easily be eliminated by the use of selective plates to support the growth on the filter (Jagow & Hill, 1988).

The colony hybridisation technique has never gained much use for direct detection of pathogens in food under routine conditions. One reason is that signals tends to be much less clear when non-radioactive labelling of probes is used, which must be considered a prerequest when used in routine laboratories. Insufficient detection limit when the sample is not a liquid also plays an important role. In order to be spread on the surface of the plate, a ten-fold dilution of the sample must be

Table 3
Examples of DNA-probes for detection of food-borne pathogenic bacteria

Bacteria	Probe derived from[a]	Reference
Campylobacter jejuni	a: Cryptic DNA	(Taylor & Hiratsuka, 1990)
	b: 16S rRNA sequence	(Romaniuk & Trust, 1989)
Clostridium perfringens	b: Phospholipase C	(van Damme-Johgsten, Haagsma & Notermans, 1990)
E. coli ETEC	a: LT and ST-genes	(Moseley et al., 1980)
	b: LT and ST gene	(Olsvik, Wasteson, Lund & Hornes, 1991)
E. coli VTEC	a: Verotoxin-genes	(Thomas, Smith, Willshaw & Rowe, 1991)
	b: Verotoxin gene	(Thomas et al., 1991)
E. coli EPEC	a: Plasmid borne adhesion factor	(Nataro, Scaletsky, Kaper, Levine & Trabulsi, 1985)
	b: Adherence factor	(Jerse, Martin, Galen & Kaper, 1990)
Listeria monocytogenes	a: Haemolysin gene	(Chenevert, Mengaud, Gormeley & Cossart, 1989)
	b: Invasion associated gene	(Bohnert et al., 1992)
Salmonella	a: Cryptic DNA	(Olsen, Aabo, Nielsen & Nielsen, 1991)
	b: Cryptic DNA	(Olsen, Aabo, Rasmussen & Rossen, 1995)
Staphylococcus aureus	b: Enterotoxins A,B,C,E	(Ewald, Heuvelman & Notermans, 1990)
Vibrio cholerae	a: Cholera toxin A-subunit	(Wachsmuth et al., 1993)
	b: Cholera toxin A-subunit	(Wright, Guo, Johnson, Nataro & Morris, 1992)
Vibrio vulnificus	a: Cytotoxin	(Morris Jr, Wright, Roberts, Wood, Simpson & Oliver, 1987)
	b: Cytotoxin	(Wright, Miceli, Landry, Christy, Watkins & Morris, 1993)
Yersinia enterocolitica	a: Virulence plasmid	(Hill, Payne & Aulisio, 1983)
	b: invasion associated gene	(Feng, 1992)

[a] polynucleotide probe; b: oligonucleotide probe. For the sequence, the reader is referred to the reference given.

made, and even if this was not the case, a detection limit of 1–10 CFU per 25 gram cannot be obtained.

As a means for culture confirmation test, however, colony hybridisation offers several advantages as it is possible to screen a large number of enrichment broths on one filter and process many filters in the same hybridisation solution (Kaysner, Weagant & Hill, 1988). To screen out negative broths in this matter is very cost effective. Colony hybridisation assays have been demonstrated to be equally good or superior to biochemical and serological methods in terms of sensitivity and specificity for confirmation of *Salmonella* and *Listeria monocytogenes* positive cultures (Flowers, Mozola, Curiale, Gabis & Silliker, 1987; Kim, Swaminathan, Cassaday, Mayer & Holloway, 1991). The DNA-based procedures have a specific advantage compared to the culture method in cases of atypical phenotypic characteristics. As an example, a commercial *Salmonella* DNA-detection system (Gene-Trak) was able to identify flour products spiked with a lactose positive *Salmonella strain* (C1:Z29). This strain was missed by the culture procedure in most of the laboratories included in the investigation (Sall, Lombardo, Sheridan & Parson, 1988). Another advantage with all DNA-based detection methods is the ability to separate potentially virulent bacteria from avirulent members of the same species by targeting virulence associated genes. This has been the major factor driving the development of DNA probes, as is evident from the choice of genes for DNA-probes (Table 1), and is still the major reason for the application of colony hybridisation.

In summary, colony hybridization has too high a detection limit to be useful for direct detection of pathogenic bacteria in food. As a culture confirmation test and for typing of isolates with respect to virulence genes, it is, however, a powerful technique, and it should be considered among the battery of possible methods, when there is a need to perform such investigations, even at a routine level. In recent years, PCR, which holds some of the same characteristics as colony hybridization, but which is less labour consuming and holds the potential for automation, have taken over for these applications in many laboratories that use such methods.

4. The use of single phase DNA-based detection methods in food microbiology

As mentioned above, rRNA directed oligonucleotide probes have been preferred for single phase hybridisation assays. This assay format, in terms, have been the choice for the vast majority of commercially available DNA-detection assay for use in food microbiology. The methods have performed well in comparison with traditional methods for culture confirmation, but like colony hybridization, they have not gained intense use in routine laboratories. Below a short description of their performance will be given with emphasis on comparative testings.

The first *Salmonella* directed assay (colorDNAH) did not detect members of *S. bongori*. In a comparative study with ELISA methods, the assay cross-reacted to *Citrobacter freundii*, but showed consistently less false positive than the two ELISA kits tested. The trial, however, showed that enrichment conditions have to be

selected carefully, as the detection limit was as high as 10^8–10^9 CFU/ml for some *Salmonella* strains (Curiale, Klatt et al., 1990). Probes detecting *S. bongori* were later included in this assay, and in a comparative study on artificially inoculated and naturally contaminated food samples, the revised colorDNAH assay did not perform significantly different from the standard BAM/AOAC culture procedure (Wilson et al., 1990). This conclusion was supported by collaborative studies reported by Curiale, McIver, Weathersby and Planer (1990) and Rose, Llabres and Bennett (1991).

Less convincing results were obtained with a detection system directed against 16S rRNA of thermophilic *Campylobacter*. When this system was used to detect the thermophilic *Campylobacter* in water washes from 38 broiler carcasses, sensitivities and specificities of only 70 and 66% were obtained (Tenover, Carson, Barbagallo & Nachamkin, 1990).

A non-radioactive oligonucleotide assay has also been developed to detect *Listeria monocytogenes* in food, but the assay cannot separate *L. monocytogenes* from other members of the genus *Listeria*. When used as a culture confirmation test on enrichment broths from 306 food samples, the assay had an apparent false positive rate of 2.9% and a false negative rate of 4.7% (King, Raposa, Warshaw, Johnson, Halbert & Klinger, 1989). On naturally contaminated samples, the assay performed at least as good as culture methods and outperformed an ELISA method for detection of *Listeria* in raw milk samples (King et al., 1982; Rodrigues, Gaya, Medina & Nunez, 1993).

The commercial methods in general have performed well for culture confirmation and are simple to perform. They are not widely used. The reason for this must be assumed to be a combination of cost, impractical protocols in relation to normal working routines, and a degree of conservatism among the users. In research in food microbiology, rRNA derived oligonucleotide probes have recently gained renewed interest due to their application in in situ hybridization. By use of this technique, detailed studies of both bacterial physiology during e.g. inactivation in food (Tolker-Nielsen & Molin, 1996) and in situ studies of interaction between pathogenic bacteria and food or animals (Poulsen, Lan, Kristensen, Hobolth, Molin & Krogfelt, 1994) can be performed.

5. The use of PCR in food microbiology

PCR-based techniques are used increasingly in research in food-microbiology, because they offer a sensitive and specific detection of pathogens, and, like the hybridization methods, can separate virulent bacteria from avirulent members of the same species. Very reliable methods have been developed for the relevant pathogenic bacteria (Table 4), but still the use in routine analysis is modest. As will be evident from the description below, this is not due to lack of good PCR-methods but to lack of methods that can release the pathogenic bacteria from the food matrix into a suitable small test volume.

Direct detection of organisms in food samples is a major goal for PCR technology, but this is difficult to obtain. The PCR-method can produce a positive reaction from nucleic acids from one organism, but the small volume of 5–10 μl culture or sample that can be used in one reaction sets the detection limit as high as approximately 10^3 cells per ml. Direct detection by PCR has, therefore, only been performed in very few cases.

Table 4
Examples of PCR-methods for detection of food-borne pathogenic bacteria

Bacteria	PCR method targetted towards	Reference
Bacillus cereus	Lecithinase gene	(Schraft & Griffiths, 1995)
Campylobacter jejuni and *C. coli*	Flagellin genes	(Rasmussen et al., 1996)
	16S rRNA	(van Camp, Chapelle & de Wachter, 1993)
Clostridium perfringens	Toxins	(Harvard, Hunter & Titball, 1992)
Cl. botulinum	Toxins	(Szabo, Pemberton & Desmarchelier, 1995)
E. coli	Toxin genes and adhesion factors (multiplex)	(Franck et al., 1998)
Listeria monocytogenes	Invasion associated gene	(Bubert, Köhler & Geobel, 1992)
	Haemolysin gene	(Fluit, Torensma et al., 1993)
Salmonella	Crytic DNA fragment	(Aabo et al., 1993)
	Flagellin genes	(Luk, Kongmuang, Reeves & Lindberg, 1993)
Shigella dysenteriae and/or *flexneri*	Invasion associated genes	(Lampel, Jagow, Trucksess & Hill, 1990) (Islam & Lindberg, 1992)
Staphylococcus aureus	Enterotoxins	(Becker et al., 1998)
Yersinia enterocolitica	Invasion associated gene	(Rasmussen et al., 1994)
	Enterotoxin	(Ibrahim, Liesack & Stackenbrandt, 1992)
Pathogenic vibrio (*V. cholerae* O1, *V. parahaemolyticus*, *V. vulnificus*)	Toxin genes (muliplex)	(Trost et al., 1993)

By a combination of washing in PBS and filtrations and centrifugations, Wang, Cao and Johnson (1992) were able to detect 4–10 CFU per food sample in one trial, while a retrial failed to confirm the result. Kock, Payne, Wentz and Cebula (1993) used primers that amplify a 779 bp region from the toxin genes *ctxA* and *ctxB* to detect Vibrio cholerae organism in various food samples by direct PCR. Positive results were seen with seeded fruits and vegetables. Seeded shellfish homogenates (10% blends of oyster or shrimp) often inhibited the PCR reaction but the inhibition could be removed by reducing the proportion of oyster or shrimp homogenate to 1%. Oysters seeded with as few as 10 CFU/g produced the amplicon of the correct size after an 8-h enrichment, and the combination of a shortened enrichment procedure and PCR is by far the most used set up for PCR methods in food microbiology.

The potential of PCR methods for culture confirmation is interesting in light of the possibilities for performing automatic PCR. That PCR may be at least as sensitive and often more sensitive than traditional culture and biochemical methods for culture confirmation have been documented on several occasions. The sensitivity of a PCR-based culture confirmation test was estimated to be 0.92 when tested for detection of *Salmonella* in 96 naturally contaminated samples of minced meat, while the traditional method missed many positive samples due to the heavy indigenous flora and only showed a sensitivity of 0.50 (Aabo, Andersen & Olsen, 1995). The same PCR method (Aabo, Rasmussen, Rossen, Sørensen & Olsen, 1993) was used in the development of a true 24 h PCR based detection method, employing only 6 h pre-enrichment before the DNA detection and with a detection limit of approximately 50 CFU/reaction (Gouws, Visser & Brözel, 1998). Segments of the tandem arranged flagellin genes in *Campylobacter* were used as target in a PCR methods which detected *C. coli* and *C. jejuni*. In this assay, the labelled PCR products were immobilized to microtiter wells and hybridized with an oligonucleotide internal in the correct PCR-fragment. Chicken faeces and rectal swabs from chickens were investigated. The culture method and the PCR showed 100% agreement for culture confirmation (Rasmussen, Olsen, Jørgensen & Rasmussen, 1996). Full agreement was likewise observed in a comparison of traditional methods and PCR for the detection of *L. monocytogenes* in enrichment cultures from milk, meat, ice-cream, sausages and chicken (Bohnert, Dilasser, Dalet, Mengaud & Cossart, 1992).

Many sorts of food contain substances that are inhibitory to PCR, and partial or total inhibition may also be encountered due to compounds in selective media or chemicals from DNA extraction procedures (Rossen, Nørskov, Holmström & Rasmussen, 1992; Wernars, Heuvelman, Chakraborty & Notermans, 1991). Development and application of extraction methods that concentrate the target organisms in a small volume, and which remove the target bacteria from the inhibitory substances, is therefore a high priority. The simplest approach to remove inhibitory substances is by dilution of the sample (Rossen et al., 1992), but this procedure lowers the sensitivity of the method. Another simple way is to capture the bacteria by antibody coated magnetic beads [immunomagnetic separation technique (IMS)] and transfer the beads to a PCR friendly environment. A *L. monocytogenes* specific PCR-methods was tested in combination with this principle. Roughly, a 10-fold better detection levels could be seen due to the fact that the magnetic beads concentrates the bacteria in a smaller volume; however, the authors estimated the increase in detection level that could be attributed to removal of inhibitory substances to be much more dramatic based on comparisons of PCR performed with PBS and enrichment broths (Fluit, Torensma et al., 1993). Widjojoatmodjo, Fluit, Torensma, Keller & Verhoef, 1995 used *Salmonella* specific antibody-coated magnetic particles to concentrate bacteria in a magnetic immunopolymerase chain reaction assay (MIPA). On spiked samples of chicken meat the detection limit of direct PCR was 10^7 CFU/g corresponding to a 100-fold inhibition of the PCR assay compared to detection on pure cultures, while 10^5 CFU/g was detected when the MIPA technique was used directly on the spiked samples. Improvements were obtained with preenrichments of 6 and 24 h before MIPA (Fluit, Widjojoadmodjo, Box, Torensma & Verhoef, 1993). A two-step PCR method for detection of *Y. enterocolitica* has been developed based on the sequence of the *inv* locus (Rasmussen, Rasmussen, Andersen & Olsen, 1994). When applied in connection with IMS to detect *Y. enterocolitica* from tonsil swabs of naturally infected pig carcasses, direct IMS-PCR only detected 60 out of 164 positive samples. DNA extraction from the swab samples did not improve this, while the IMS-PCR method showed better sensitivity than the traditional method when a preenrichment step was included (Rasmussen et al., 1995).

Heat treated food may contain dead or damaged cells with no relevance for food safety, but which may create positive signals in a PCR-reaction due to the stability of the DNA-molecules. The so-called viable but non-culturable bacteria have also been reported to give positive reactions by PCR. *V. vulnificus* form viable but non-culturable cells when held for several weeks under poor nutrient conditions. Such cells could be detected by PCR, but detection sensitivity was inexplicably low (Brauns, Hudson & Oliver, 1991), possibly because the DNA undergoes changes that makes it difficult to amplify. For the time being, enrichment procedures are included with most PCR-methods, and hence only culturable organisms will be detected.

Under research conditions, the PCR-products are often scored by gel electrophoresis, but this procedure is

not suitable for routine purposes. A procedure termed DIANA, for detection of immobilized amplified nucleic acid (Uhlén et al., 1990), was tested with a PCR for verotoxin producing *E. coli* (Olsvik, Rimstad et al., 1991; Olsvik & Strockbine, 1993). In this system, part of the lac operon (*lacO*) is introduced into one primer, and following the PCR-reaction, the lac-inhibitor protein lacI fused with a suitable enzyme is bound to the gene to give a substrate induced signal (Lundeberg, Wahlbert & Uhlén, 1990). The same system was tested with a PCR-method for detection of *Y. enterocolitica*, and approximately 1–3 bacteria per assay corresponding to 10–30 CFU/g of food sample or per 100 ml of surface water were detected (Kapperud, Vardung, Skjerve, Hornes & Michaelsen, 1993). In another system, 11-dUTP-digoxigenin is incorporated into the PCR product during amplification to facilitate detection, and biotin labelled primers are used to enable capture of the labelled products with streptavidin-coated magnetic beats. The detection limit has been shown to be 1 template per PCR reaction with this system (Holmstrøm et al., 1993).

Several pathogens can be detected simultaneously in one step by multiplex PCR. Such multiplex methods of relevance to food microbiology have been used to detect variants of enteropathogenic *E. coli* (Franck, Bosworth & Moon, 1998), to detect the three pathogenic *Vibrio*, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* (Trost, Hill, Kaysner & Wekell, 1993), to perform species differentiation within *Listeria* (Bubert, Köhler & Goebel, 1992), to characterize strains of *S. aureus* with respect to enterotoxins (Becker, Roth & Peters, 1998) and to simultaneously detect *E. coli*, faecal coliforms, *Salmonella*, and pathogenic Vibrio (Brasher, DePaolo, Jones & Bej, 1998). To the extreme, a common enrichment broth and a common PCR-reaction mix were used in a multiplex PCR, where 13 different food borne pathogenic bacteria were detected in the same reaction (Wang, Cao & Cerniglia, 1997). Such methods show the potential for practical every day use of PCR methods in food microbiology.

In summary, PCR methods are well developed and when applied as culture confirmation tests, they are reliable, fast and sensitive. The ability to concentrate the target organisms from food without co-concentration of inhibitory substances still need to be improved before detection directly from processed food becomes a realistic goal.

6. Oligonucleotide arrays (DNA-chips)

DNA chips consist of oligonucleotide arrays bound to a solid support in a systematic manner which enables hybridization results to be scored automatically. Usually, glass forms the solid support and labelling of probes is most conveniently done by use of a fluorescence label. A CDC-scanner or a scanning confocal microscope is used to capture the image. A simplified drawing of the basic principle in DNA-disc based hybridisation is shown in Fig. 3. Two systems are in use, one in which short oligonucleotides have been synthesized directly on the solid support (Chee et al., 1996) and one in which short DNA molecules are synthesized and then fixed on a suitable support using robotics (Shena, 1996). A detailed description of the technological developments behind the DNA-chips has been given elsewhere, and the reader is referred to these papers (Marshall & Hodson, 1998; Ramsay, 1998; Southern, 1996).

DNA chips are now commonly used to analyse DNA sequences. The resolution is high, and by comparison of DNA sequences from organisms of the same species, biologically significant point mutations can be detected in the same step as a species specific identification is performed. As an example, the *rpoB* gene diversity in rifampin resistant *Mycobacterium tuberculosis* has been compared by chip technology. Species specific identification of non-tubercular *Mycobacterium* was possible and for *M. tuberculosis*, variation in *rpoB* gene gave additional information as to sub-populations (Gingeras et al., 1998). As the number of fully sequenced organisms keeps growing, more putative genes are recognized and sequences from these genes can be incorporated into ready made DNA-discs. By hybridization analysis based on cDNA of mRNA it is possible to study the expression of many genes simultaneously, greatly enlarging our understanding of the way organisms react towards environmental changes. This was first performed

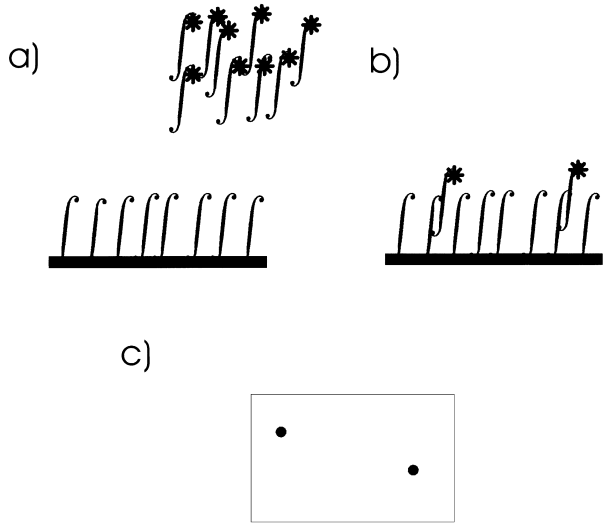

Fig. 3. A simplified presentation of oligonucleotide array hybridisation (DNA-disc). In relation to food microbiology, the obvious assay format will consist of a solid support, usually made from glass, on which probes for the relevant pathogenic bacteria are fixed in known positions (a). Sample DNA is processed to consist of fluorescence-labelled, short oligonucleotides and are hybridised to the oligonucleotides on the disc (b). The disc is scanned and the result is scored by a CDC-camera or scanning confocal microscope (c).

for studies of eucaryotic cells (DeRise et al, 1996; DeRise, Iyer & Brown, 1997) but has recently been used to study gene expression in the bacterium *Streptococcus pneumoniae* (De Saizieu, Certa, Warrington, Gray, Keck & Mous, 1998). In the latest development of the technique, double stranded DNA chips have been constructed and used to study DNA-protein interactions (Bulyk, Gentalen, Lockhart & Chruch, 1999).

In relation to the detection of food-borne pathogenic bacteria, there are still many obstacles to be overcome. The potential is, however, almost revolutionary. While traditional DNA-hybridization works with one probe at a time, thousands of probes can go into the same chip-based hybridization (Braxton & Bedilion, 1998). This leaves room for probes for all food-borne pathogenic bacteria, for subtyping of each bacteria for epidemiological purposes, for their common antibiotic resistance genes with a view to antibiotic resistance surveillance, and then still room for genes of specific interest. DNA-chips, however, faces some of the same problems that have met the other DNA-methods. It will still be a problem to capture the relevant bacteria from the food matrix in sufficient concentration to generate the labelled probes needed to produce a signal. As a starting point it must therefore be envisaged that the technology will be introduced as an ''all-in-one''-culture confirmation test.

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