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USE OF DNA PROBES IN THE DIAGNOSIS OF INFECTIOUS DISEASE

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ABSTRACT

DNA probes specific for bacterial or other viral pathogens can be used in DNA hybridization assays to detect the presence of the pathogen in food, stool, urine, or other complex samples. Such assays are faster and less expensive than traditional microbiological culture techniques, and in many applications can compete with assays based on monoclonal antibodies.

INTRODUCTION

DNA hybridization technology allows the detection of potentially any organism of interest in tissue biopsies, stools, urine, foods, or other complex samples. Probe DNA sequences can be obtained from a variety of sources, including plasmids, virulence genes, toxin genes, viral genomes, or organelle nucleic acids. Moseley et al.[1] have used genes encoding enterotoxins to detect enterotoxigenic E. coli in isolated colonies and directly in stools from patients with acute diarrhea. Wirth and Pratt[2] used kinetoplast DNA to detect Leishmania species in cutaneous tissues. A variety of viral pathogens have been studied using this methodology, including hepatitis B virus[3], cytomegalovirus[4], and Epstein-Barr virus[5]. Enterotoxigenic E. coli and Yersinia enterocolitica have been detected in foods using similar probes[6,7,8].

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The hybridization assay used in most of examples is a two phase system, with usually the probe DNA immobilized on a solid matrix, and the other DNA in solution. Typically, one begins with a complex sample such as a food or stool culture, or with suspensions of food, stool, or other tissue samples or body fluids, and then collects the bacteria or viruses or other microorganisms onto a solid matrix, usually nitrocellulose or nylon filters. This collection can be done by filtration of the sample through the nitrocellulose, in which case the microorganisms are retained on the filter, or one can spot the sample directly onto the filter. It is then necessary to lyse the bacteria or other organisms to release the DNA. The DNA duplex must then be denatured into separate strands and bound to the nitrocellulose.

In the next step the DNA probe is added to the system. The probe sequence seeks out complementary sequences in the DNA attached to the filter, and thereby forms a hybrid in those regions. The hybrids formed can be detected by assaying a reporter molecule attached to the probe DNA. This reporter molecule can be as simple as a ^{32}P radiolabel, or as complex as an enzyme or hapten conjugated to the DNA. In the case of a radiolabel, hybrids are easily detected by simply counting the filter in a scintillation counter, or by developing an autoradiogram.

A number of investigators have attached biotin to the DNA probe, which is assayed after hybridization using a reagent consisting of avidin and an enzyme or some other molecule[9-13]. The biotin can be incorporated as a biotinylated nucleotide during nick-translation of the probe DNA. After hybridization, an avidin-enzyme reagent is added, which then reacts with the biotin in the DNA. Addition of the enzyme substrate allows a colorimetric assay of hybridization. It is also possible to directly modify the DNA probe with a hapten, which is then assayed through the use of an antibody which reacts with that hapten. Enzymes can also be directly linked to the DNA, and then assayed by addition of substrate[14].

SPECIFIC DNA PROBES FOR SALMONELLA

Currently the food industry tests its products for the presence of one bacterial pathogen in particular. This organism, Salmonella, causes severe diarrhea that lasts 3 to 5 days. To control Salmonella infection, manufactured food products and raw materials for food products are tested for the presence of Salmonella. We estimate that there are at least five million tests performed per year in the domestic food industry, and that the worldwide market is two to three times as large. The testing procedure commonly used is a microbiological assay that takes five to seven days to complete. During this time, food manufacturers typically hold both their raw materials and finished goods in inventory until they are shown to be free of Salmonella contamination. In the event that foods are released to the public, but later found to contain Salmonella, recalls of the products are both expensive and undesirable. In larger food companies Salmonella testing is usually done in-house by a quality control department. Some large companies as well as many of the smaller companies have Salmonella testing performed by an independent testing laboratory. In the latter case, the average cost of this assay to the food manufacturer customer is $10-15 per test.

The microbiological method used to detect Salmonella is outlined in Table I. Typically foods are inoculated into media specified by the Association of Official Analytical Chemists, which usually involves a 25g or 400g food sample cultured into non-selective, or pre-enrichment, media. These cultures are allowed to grow for 24 hrs, and then are subcultured into selective, or enrichment, media for another 24 hour period. After that step, the cultures are plated onto selective media for 24 to 48 hours. Any suspect Salmonella colonies are then picked from these selective agar plates, and are then screened in further biochemical tests. Any isolates that appear to have the fermentation pattern of Salmonella are serotyped for final confirmation[15].

A DNA hybridization test, however, can be done in much less time. As one can see in Table I, the initial pre-enrichment is

TABLE I

Comparison of the Standard Microbiological Assay with a DNA Hybridization Assay for *Salmonella* spp. in Foods

Microbiological Assay	DNA Hybridization Assay
1. Pre-enrichment (24 Hr)	1. Pre-enrichment (24 Hr)
2. Selective enrichment (24 Hr)	2A. Selective enrichment (6-24 Hr)
	B. Hybridization assay (4 Hr)
3. Selective plating (24-48 Hr)	
4. Biochemical screening (24 Hr)	
5. Serology	

still required, because the numbers of salmonellae in the food sample are often so small. After that 24 hour period, the cultures are then subcultured for a short selective enrichment of 6 hours, and then the DNA hybridization assay is performed. This assay takes about four hours, which means that the total time required to determine the presence of *Salmonella* in the food is less than 48 hours.

Finding the appropriate sequences to use as probes is often a complex problem. For example, an organism such as *Salmonella* does not have well-defined toxin genes or virulence factors such as those described for *E. coli* or *Y. enterocolitica*, nor can one find a widely distributed plasmid or set of plasmid genes that might serve as probes to detect all *Salmonella* spp. In this instance, it was necessary to search the *Salmonella* genome for DNA sequences which empirically serve as good probes. That this is possible to find a chromosmal sequence that is specific to *Salmonella* and is found in all *Salmonella* spp. is rather surprising, since so much of the *Salmonella* genome shares homology with closely related bacteria, such as *E. coli* and *Citrobacter*. However, we were able to isolate approximately 1% of the total DNA

TABLE II

Characterization of Salmonella-specific Probes

Probe	Length[1]	Distribution[2]
RF321	4.9KB	100%
RF356	4.1KB	100%
RF319	6.0KB	95%
RF333	5.7KB	95%
RF305-1	3.6KB	95%
RF304	2.3KB	50%
RF344	4.8KB	50%
RF347-3	1.4KB	50%
RF318	3.2KB	50%
RF367	3.2KB	50%

[1]Length measured in kilobase pairs

[2]Distribution calculation based on hybridization study of 352 Salmonella isolates

in Salmonella typhimurium that appears to be unique to the genus Salmonella.

For any probe sequence it is necessary to demonstrate two properties: that of exclusivity and inclusivity. In this instance this was accomplished by screening large panels of non-Salmonella bacteria, to demonstrate that only Salmonella will hybridize, and large panels of Salmonella isolates, to demonstate that all salmonellae can be detected.

A total of ten unique clones were isolated and are shown in Table II. The ten clones shown fall into three categories: those that hybridize to 100% of all the Salmonella isolates tested, those that hybridize to less than 100% but greater than 95%, and those that hybridize to 50% or less. Clones RF321 and RF356

hybridize to 100% of all the Salmonella islates tested; clones RF333, RF319, and RF305-1 hybridize to greater than 95% but less than 100%, and the remaining five clones in Table II hybridize to less than 50%. The Salmonella isolates used for these determinations are listed elsewhere[16].

These probes have been used in DNA hybridization assays with spiked foods in the development of a DNA hybridization test for the presence of Salmonella[17], and in field studies with naturally contaminated foods to compare the efficacy of DNA hybridization with the traditional microbiological methodology[18].

SUMMARY

It is possible to isolate specific DNA sequences for important human pathogens such as Salmonella, and successfully use these as DNA probes to assay the presence of the pathogen in a complex sample such as a food culture. This methodology is easily extended to other organisms in both foods and clinical samples. Further development work, however, is necessary to adapt nonisotopic labeling techniques and hybridization procedures to a fast and easy system for handling contaminated food samples.

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