DNA Probes for the Identification of Pathogenic Foodborne Bacteria and Viruses

Keith A. Lampel, Peter Feng, and Walter E. Hill

Foodborne disease in the United States costs billions of dollars annually because of increased morbidity and mortality, time lost in the workplace, and reduced productivity (Archer and Kvenberg 1985). Because outbreaks of foodborne illnesses may be underreported by as much as a factor of 30 (Hauschild and Bryan 1980), the number of cases of gastroenteritis associated with food was estimated to be between 68 million and 275 million per year (Archer and Kvenberg 1985). Even at the lower end of this range, foodborne disease would be a major public health problem. From 1983 through 1987, 2397 outbreaks (54,453 cases) of foodborne illness were reported to the Centers for Disease Control (Bean et al. 1990). Of these, the causative agent was confirmed in only 910 cases (38%).

To reduce the adverse impact on public health caused by foodborne microbial disease, we must have methods to identify foodborne pathogens quickly. During the past decade, several rapid methods for the identification of foodborne bacteria have been developed. Many of these tests require pure cultures to determine the physiological, biochemical, or immunological characteristics of the bacteria; however, such methods usually cannot identify pathogenic strains. Research on bacterial virulence factors is now providing information on the mechanisms by which some of these bacteria cause disease in human hosts. It is possible, then, that tests may be devised to determine whether the bacteria are pathogenic, based on the genetic information they carry.

Bacteria that are the principal causes of foodborne disease include strains of Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Salmonella
spp., Staphylococcus aureus, Shigella spp., Vibrio cholerae, Vibrio para- 
haemolyticus, Vibrio vulnificus, Yersinia enterocolitica, and Yersinia pseu-
dotuberculosis (Doyle 1985; Archer and Young 1988; Doyle 1989; Ryster 
and Marth 1989). Gene probes have been developed to identify these orga-
nisms as well as a number of viruses that cause foodborne disease.

GENE PROBES: DEVELOPMENT AND USE

Gene probes are nucleic acid molecules (usually DNA) of known genetic 
specificity. They are labeled to be easily identified, and are used to deter-
mine the presence of specific nucleotide sequences. How probes are devel-
oped and used, as well as technical considerations regarding labels, sensitiv-
ity, and specificity, are the major topics of this chapter.

How Probes Work

DNA hybridization has been known for more than 20 years (McCarthy 
1967; Britten and Kohne 1968), but only within the last decade have ad-
vances in recombinant DNA techniques made it possible to prepare signifi-
cant quantities of specific DNA fragments for use as gene probes. The key 
features of the DNA molecule that permit probes to function are comple-
mentarity of the two strands of polynucleotides that constitute the double 
helix and the sequence of nucleotides that encode genetic information. Two 
organisms that are closely related in evolutionary history will share many 
similar nucleotide sequences.

The strands of the DNA double helix can be separated by heat or alkaline 
treatment. If the strands of the probe DNA and the target DNA are comple-
mentary, they will form a double-stranded molecule when cooled or when 
the pH is lowered. Double helices composed of strands from different 
sources are designated as hybrids; the process of their formation is called 
DNA hybridization. Hybrids may also be formed between strands of RNA 
and DNA. To favor probe–target hybrids, the amount of probe should be 
in excess of the amount of target.

Development of Probes

Before a gene probe is developed, a specific target must be selected, for 
example, a particular bacterial genus in a food or a certain pathogenic 
strain. Gene probes for identifying an entire taxonomic group are often 
targeted to evolutionarily conserved genes, such as those encoding ribo-
somal RNA, whereas to identify a particular pathogenic strain, the usual 
target is a variant gene that encodes a virulence factor such as a toxin.