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Detection of Food-Borne Pathogens Using Oligonucleotide Microarray

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A method for the rapid detection and identification of food-borne pathogens is needed by the food industry, food safety agencies, and public health related organizations. Major food-borne pathogens include *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium. Accurate and timely detection of food-borne pathogens is paramount for the prevention of a food-borne epidemic, and thus any screening method must be sensitive and specific, as well as rapid. Classical diagnostic methods generally require at least 2~3 days from culturing and biochemical identification. Several nucleic acid-based methods have been developed for the rapid and simultaneous detection of multiple pathogenic bacteria [1]. PCR is the most commonly applied method. However, DNA-based microarray technology has been used for genomic studies including gene expression, genotyping and single-nucleotide polymorphisms, and could easily be applied to the detection of food-borne pathogens. In addition, microarray technology has the potential for high-throughput detection of multiple pathogens in one experiment and has been suggested as a means for the specific detection of food-borne pathogens after multiplex PCR.

Recently, cDNA and oligonucleotide microarray technology has been applied for the analysis of microbial pathogens and a single-chip multipathogen oligonucleotide microarray was constructed for the simultaneous analysis of food-borne pathogens. However, in these studies, the investigators used probes based only on a few virulence genes and 16S or 23S rRNA genes [2]. These sequences are often shared among closely related bacteria and can generate cross-hybridization reactions. In addition, because multiplex PCR has a maximum number of primers that can be used in a single reaction, relying on PCR amplification prior to hybridization limits the number of probes or target bacteria that can be tested at one time, a significant disadvantage in the multidetection of pathogens.

Genome sequence of 675 microorganisms are publicly available at NCBI (April 6, 2008) and the

Table 1. Genomic DNA sequences of various bacteria used in this study

Bacterial strains	Genbank	Total base(bp)	Protein coding genes
Bacillus cereus ATCC 14579	AE016877	5,411,809	5,234
Listeria monocytogenes strain EGD	AL591824	2,944,528	2,846
Listeria inocua	AL592022	3,011,208	2,989
Staphylococcus aureus N315	BA000018	2,814,816	2,594
Staphylococcus aureus Mu50	BA000017	2,878,529	2,714
Staphylococcus aureus MW2	BA000033	2,820,462	2,632
Clostridium botulinum ATCC 3502	-	3,886,916	-
Clostridium perfringens	BA000016	3,031,430	2,660
Campylobacter jejuni NCTC 11168	AL111168	1,641,481	1,634
Vibrio parahaemolyticus RIMD	BA000031	3,288,558	3,080
Yersinia enterocolitica		4,615,899	
Escherichia coli O157:H7 EDL933	AE005174	5,528,445	5,324
Escherichia coli O157:H7	BA000007	5,498,450	5,361
Escherichia coli K12	U00096	4,639,221	4,279
Salmonella Typhimurium LT2	AE006468	4,857,432	4,451
Salmonella Typhi CT18	AL513382	4,809,037	4,395

genomes of most known food-borne pathogens are available (Table 1). In this study, we used comparative genomics to select oligonucleotide probes specific for major food-borne pathogens for use in microarray analysis [3, 4]. We analyzed the hybridization pattern of this microarray with the Cy3-labeled genomic DNA of various food-borne pathogens and other bacteria. Our microarray showed a highly specific hybridization pattern with the genomic DNA of each food-borne pathogen [5]. Microarray data were analyzed and clustered using the GenePix Pro 6.0 and GeneSpring GX 7.3.1 programs. The analyzed phylogenetic tree revealed the discriminating power of our microarray analysis in that each food-borne pathogen clustered according to its hybridization specificity and non-pathogenic species were discriminated from pathogenic species.

Our method can be applied to the rapid and accurate detection and identification of food-borne pathogens in the food industry. In addition, this study demonstrates that genome sequence comparison and DNA microarray analysis have a powerful application in epidemiologic and taxonomic studies, as well as in the food safety and biodefense fields.

References

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