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Minireview

Molecular techniques: An overview of methods for the detection of bacteria

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Several DNA molecular markers are now available for use in surveillance and investigation of food-borne outbreaks that were previously difficult to detect. The results from several sources of literature indicate substantially different degrees of sensitivities between conventional detection methods and molecular-based methods. The new technology is noted for increased sensitivity over the traditional culture methods which they complement.

Key words: molecular techniques, fingerprinting, microorganism.

INTRODUCTION

Molecular techniques are major tools for the analysis of microorganisms from food and other biological substances. The techniques provide ways to screen for a broad range of agents in a single test (Field and Wills, 1998). It has truly come of age and its range of application is perceived to broaden in the near future. The food industries, water processors, and analytical laboratories have taken up the latter method; for rapid differentiation of species, strain identification and definition of strain relatedness from infected samples.

Molecular methods varies with respect to discriminatory power, reproducibility, ease of use, and ease of interpretation (Lasker, 2002). I report here a summary of the molecular detection methods applicable to microbes from food, plant material, soil, and water.

POLYMERASE CHAIN REACTION (PCR)

PCR methods have been described in more detail by Hoelzel and Green (1998). Saiki et al. (1985) published the first experimental data on PCR, and ever since PCR technique (Mullis and Faloona, 1987) has tremendously influenced research in diverse areas of biological sciences leading to an unprecedented understanding of microorganisms. Using PCR, it is now possible to make virtually unlimited copies of a fragment of DNA (Field and Wills, 1998). The organism of interest can be detected directly through PCR assays in a much shorter time than conventional culture takes.

Campylobacter, the most common cause of acute bacterial gastroenteritis in the developed world, has been detected from meat by PCR (Cloak et al., 2001). PCR assays also allow the identification of *Lactobacillus curvatus*, *L. graminis*, and *L. sake* (Berthier and Ehrlich, 1998). Brooks et al. (1992) used PCR to amplify specific rDNA sequences of *Carnobacterium* spp. in purified DNA extracts, crude cell lysates, and food samples. An analogous PCR method has been designed for the identification of genetically engineered *L. curvatus* in raw sausage. Dahlenborg et al. (2001) used PCR based methodology to investigate the prevalence of *Clostridium*

botulinum in primary production and for monitoring the botulinum neurotoxin gene expression. Heilig et al. (2002) developed a *Lactobacillus* group-specific PCR primer, which selectively amplify 16S ribosomal DNA (rDNA) from lactobacilli and related lactic acid bacteria, including members of the genera *Leuconostoc*, *Pediococcus*, and *Weissella*. The sequences of *Leuconostoc* species (Heilig et al., 2002) retrieved have only been detected in fermented food products and never in gastrointestinal tract samples.

Theron et al. (2001) also developed a sensitive seminested PCR method for the detection of *Shigella* in spiked environmental water samples. These workers had earlier detected toxigenic *Vibrio cholerae* from environmental water samples by an enrichment broth, semi-nested PCR procedure (Theron et al., 2000). Sea water and organic material analysed (Alam et al., 2003) to determine *Vibrio parahaemolyticus*, a potentially pathogenic bacterium, showed over 22% of samples positive for *V. parahaemolyticus* than the conventional (Most Probable Number) MPN culture technique could detect.

PCR has already proven valuable in the screening of rhizobacteria for 1-amino-cyclopropane-1-carboxylic (ACC) deaminase (Babalola et al., 2003). Taylor et al. (2001) described the detection of *Erwinia amylovora* (pectolytic bacteria) in plant material using PCR. Similarly, Sánchez-Contreras et al. (2000) developed and tested four primers that recognize homologous conserved regions in the *Sinorhizobium meliloti* genome by PCR. The method was used to establish a collection of *S. meliloti* strains from soils polluted with polychlorinated biphenyls and/or polycyclic aromatic hydrocarbons a process, which could have been otherwise time-consuming. Moreover, PCR approach has identified the bacterium from nodules of *Medicago* sp. plants collected from field samples. The results are useful for identification of *S. meliloti*, especially when high numbers of other bacteria are expected to be present in nodules.

The development of multiple assays such as multiplex PCR means that several bacterial species can be identified in a single assay (Field and Wills, 1998). For example, a single multiplex PCR has been used to detect *Salmonella*, *Campylobacter*, *Shigella* species and *E. coli* in faecal samples (QIAGEN, 2001).

DNA AMPLIFICATION FINGERPRINTING (DAF) and RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

DAF and RAPD are amplification-based nucleic acid fingerprinting techniques (concurrent detection of multiple loci without assignment of a genotype) that use an in vitro enzymatic reaction to specifically amplify a multiplicity of target sites in one or more nucleic acid molecules (Micheli et al., 1994). The amplification reaction is generally driven by short synthetic oligonucleotides of arbitrary or semi-arbitrary sequence, that produce a collection of amplified products of largely non-allelic nature. DAF uses a single primer (5-10 bp) to amplify genomic DNA at random. *Salmonella enterica* serotype Typhimurium, obtained from human, animal (clinical), and food sources, were typed by DAF (Daly et al., 2000). Data from DAF and their other studies indicate a remarkable degree of homogeneity at a molecular level among contemporary isolates of *S. enterica* serotype Typhimurium DT104.

The RAPD technique was first employed by Williams et al. (1990) to examine human DNA samples from anonymous individuals. Since then several authors have reported on the application of RAPD technique in microorganisms (e.g. Babalola, 2002). It uses random primers (Williams et al., 1990) and can be applied to any species without requiring any information about the nucleotide sequence. The amplification products from this analysis exhibit polymorphism and thus can be used as genetic markers. The presence of a RAPD band, however, does not allow distinction between hetero- and homozygous states. The fragments are scored as dominant Mendelian elements, and the protocols are relatively simple.

Nowrouzian et al. (2001) designed a RAPD typing method for the identification of *E. coli* strains in the normal human intestinal microflora. The band pattern generated in the analysis represents genome characterization of a particular bacterial strain (Welsh and McClelland, 1990). In addition, the method has the potential for analyzing phylogenetic relationships among closely related species (Williams et al., 1990) and can distinguish between strains within a species.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs)

The procedures involve isolation of DNA, digestion of DNA with restriction endonucleases, size fractionation of the resulting DNA fragments by electrophoresis, DNA transfer from electrophoresis gel matrix to membrane, preparation of radiolabelled and chemiluminiscent probes, and hybridisation to membrane-bound DNA.

RFLP fingerprinting technique is regarded as the most sensitive method for strain identification and several bacterial strains have been widely studied using this technique. Kabadjova et al. (2002) established a rapid PCR-RFLP-based identification scheme for four closely related *Carnobacterium* species (*C. divergens*, *C. piscicola*, *C. gallinarum*, and *C. mobile)* that are of interest to the food industry. Three isolates previously incorrectly identified as *C. divergens* (INRA 508, INRA 586, and INRA 515) were reclassified as *C. piscicola*. Similarly, four isolates deposited as *C. piscicola* (INRA 545, INRA 572, INRA 722, and ENSAIA 13) were reclassified as *C. divergens* based on the patterns

obtained by the 16S-23S ISR-RFLP methods.

Wang et al. (2000) and Penrose et al. (2000) proved the role of PCR and Southern hybridisation in assessing the effect of introducing 1-aminocyclopropane-1-carboxylic acid deaminase genes on disease-suppressive capabilities of *Pseudomonas fluorescens* strain CHAO. One of their results suggested that the constructed stains could be developed as biosensors for the role of ethylene in plant diseases. Manceau and Horvais (1997) used RFLP analysis of rRNA operons to assess phylogenetic diversity among strains of *Pseudomonas syringae* pv. tomato. They successfully established the close relationships existing between *P. syringae* and *P. viridiflava* species. However, the findings of Lu et al. (1996) suggested that PCR-based multiple-loci marker techniques (RAPD, AFLP, microsatellite and inter-SSR PCR) could replace RFLP in the estimation of genetic diversity.

AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLPs)

Amplified fragment length polymorphism (AFLP) analysis was developed by a team led by Marc Zabeau at Keygene N.V., Wageningen, The Netherlands (Vos et al., 1995; Zabeau and Vos, 1993). Vos et al. (1995) had described the principle of AFLP fingerprinting technique. AFLP is a variation of RAPD, able to detect restriction site polymorphisms without prior sequence knowledge using PCR amplification for detection of restriction fragment (Blears et al., 1998; Mueller and Wolfenbarger, 1999; Vos et al., 1995; Zabeau and Vos, 1993). Here, the template for a PCR reaction is a restriction enzyme-digested genomic DNA. The primers contain the restriction enzyme recognition site as well as additional 'arbitrary' nucleotides that extend beyond the restriction site. The fixed portion gives the primer stability and the random portion allows it to detect many loci. Amplified products are resolved by polyacrylamide gel electrophoresis.

AFLP analysis is one of the robust multiple-locus fingerprinting techniques among genetic marker techniques that have been evaluated for genotypic characterization (Koeleman et al., 1997). Restrepo et al. (1999) used AFLP to characterize the genetic relationships between *X. axonopodis* pv. Manihotis strains. The study of Janssen et al. (1996) revealed extensive evidence for applicability of AFLP in bacterial taxonomy through comparison of the newly obtained data with results previously obtained by well-established genotypic and chemotaxonomic methods such as DNA-DNA hybrdization and cellular fatty acid analysis.

CONCLUSION

The genomic typing techniques discussed increase knowledge in microorganisms important in food-borne gastrointestinal infections, starter cultures analysis, rapid differentiation of species, strain identification, and definition of strain relatedness. Molecular-based methods are complementary to traditional methods and are revolutionizing microbial diversity, and taxonomy research and applied fields.

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