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 vary 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 Hoetzel 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...
generally driven by short synthetic oligonucleotides of (Micheli et al., 1994). The amplification reaction is target sites in one or more nucleic acid molecules enzymatic reaction to specifically amplify a multiplicity of loci without assignment of a genotype) that use an in vitro fingerprinting techniques (concurrent detection of multiple DAF and RAPD are amplification-based nucleic acid DNA AMPLIFICATION FINGERPRINTING (DAF) and 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 chemiluminescent 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 strains 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 hybridization 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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REFERENCES


