

Minireview

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

Olubukola O. Babalola

Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria. E-mail:
olubukola_babalola@hotmail.com. Tel.: +2348037035965.

Accepted 15 November 2003

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 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 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 (Bleas 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.

ACKNOWLEDGEMENT

I wish to express my thanks to Mrs Yvonne Olatunbosun for editing this manuscript.

REFERENCES

- Alam MJ, Miyoshi S, Shinoda S (2003). Studies on pathogenic *Vibrio parahaemolyticus* during a warm weather season in the Seto Inland Sea, Japan. *Environ. Microbiol.* 5:706-710.
- Babalola OO, Osir EO, Sanni AI (2002). Characterization of potential ethylene-producing rhizosphere bacteria of *Striga*-infested maize and sorghum. *Afr. J. Biotechnol.* 1:67-69.
- Babalola OO, Osir EO, Sanni AI, Odhiambo GD, Bulimo WD (2003). Amplification of 1-amino-cyclopropane-1-carboxylic (ACC) deaminase from plant growth promoting rhizobacteria in *Striga*-infested soil. *Afr. J. of Biotechnol.* 2:157-160.
- Berthier F, Ehrlich SD (1998). Rapid species identification within two groups of closely related lactobacilli using PCR primers that target the 16S/23S rRNA spacer region. *FEMS Microbiol. Lett.* 161:97-106.
- Bleas MJ, De Grandis SA, Lee H, Trevors JT (1998). Amplified fragment length polymorphism (AFLP): review of the procedure and its applications. *J. Ind. Microbiol. Biotechnol.* 21:99-114.
- Brooks JL, Moore AS, Patchett RA, Collins MD, Kroll RG (1992). Use of the polymerase chain reaction and oligonucleotide probes for the rapid detection and identification of *Carnobacterium* species from meat. *J. Appl. Bacteriol.* 72:294-301.
- Cloak OM, Duffy G, Sheridan JJ, Blair IS, McDowell DA (2001). Incidence of *Campylobacter* in Irish retail meats and a surface adhesion-PCR method for its detection from food. *Int. J. Food Microbiol.*
- Dahlenborg M, Borch E, Rådström P (2001). Development of a combined selection and enrichment PCR procedure for *Clostridium botulinum* Types B, E and F and its use to determine prevalence in faecal samples from slaughtered pigs. *Appl. Environ. Microbiol.* 67:4781-4788.
- Daly M, Buckley J, Power E, O'Hare C, Cormican M, Cryan B, Wall PG, Fanning S. (2000). Molecular characterization of Irish *Salmonella enterica* Serotype Typhimurium: detection of class I integrons and assessment of genetic relationships by DNA amplification fingerprinting. *Appl. Environ. Microbiol.* 66: 614-619.
- Field D, Wills C (1998). Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. *Proc. Natl. Acad. Sci. U. S. A.* 95:1647-1652.
- Heilig HGHJ, Zoetendal EG, Vaughan EE, Marteau P, Akkermans ADL, de Vos WM (2002). Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Appl. Environ. Microbiol.* 68:114-123.
- Hoelzel A, Green A (1998). PCR protocols and population analysis by direct DNA sequencing and PCR-based DNA fingerprinting In: Hoelzel AR, (ed) *Molecular genetic analysis of populations: a practical approach.* IRL Press, Oxford, pp201-233.
- Janssen P, Coopman R, Huys G, Swings J, Bleeker M, Vos P, Zabeau M, Kersters K (1996). Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. *Microbiology* 142:1881-1893.

- Kabadjova P, Dousset X, Le Cam V, Prevost H (2002). Differentiation of closely related carnobacterium food isolates based on 16S-23S ribosomal DNA intergenic spacer region polymorphism. *Appl. Environ. Microbiol.* 68:5358-5366.
- Koeleman JG, Parlevliet GA, Dijkshoorn L, Savelkoul PH, Vandenbroucke-Grauls CM (1997). Nosocomial outbreak of multi-resistant *Acinetobacter baumannii* on a surgical ward: epidemiology and risk factors for acquisition. *J. Hosp. Infect.* 37:113-123.
- Lasker BA (2002). Evaluation of performance of four genotypic methods for studying the genetic epidemiology of *Aspergillus fumigatus* isolates. *J. Clin. Microbiol.* 40:2886-2892.
- Lu J, Knox MR, Ambrose MJ, Brown JKM, Ellis THN (1996). Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. *Theor. Appl. Genet.* 93:1103-1111.
- Manceau C, Horvais A (1997). Assessment of genetic diversity among strains of *Pseudomonas syringae* by PCR-restriction fragment length polymorphism analysis of rRNA operons with special emphasis on *P. syringae* pv. tomato. *Appl. Environ. Microbiol.* 63:498-505.
- Micheli MR, Bova R, Pascale E, D'Ambrosio E (1994). Reproducible DNA fingerprinting with the random amplified polymorphic DNA (RAPD) method. *Nucleic Acids Res.* 22:1921-1922.
- Mueller UG, Wolfenbarger LL (1999). AFLP genotyping and fingerprinting. *TREE* 14:389-394.
- Mullis KB, Faloona F (1987). Specific synthesis of DNA in vitro via a polymerase catalysed chain reaction. *Meth. Enzymol.* 155:335-350.
- Nowrouzian F, Wold AE, Adlerberth I (2001). Computer-based analysis of RAPD (Random Amplified Polymorphic DNA) fingerprints for typing of intestinal *Escherichia coli*. *Mol. Biol. Today* 2:5-10.
- Penrose DM, Moffatt BA, Glick BR (2000). Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. *Can. J. Microbiol.* 47:77-80.
- QIAGEN (2001). Molecular diagnostics research: clinical research. Pathogenic enteric bacteria. Data provided by J. Leushner and M. Kelly, MDSMetro Laboratory Services, Burnaby, BC, Canada.
- Restrepo S, Duque M, Tohme J, Verdier V (1999). AFLP fingerprinting: an efficient technique for detecting genetic variation of *Xanthomonas axonopodis* pv. manihotis. *Microbiology* 145:107-114.
- Saiki RH, Scharf S, Faloona F, Mullis KB, Horn GT, Ehrlich HA, Arnheim N (1985). Enzymatic amplification of beta-globulin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, 230:1350-1354.
- Sánchez-Contreras M, Lloret J, Martín M, Villaceros M, Bonilla I, Rivilla R (2000). PCR use of highly conserved DNA regions for identification of *Sinorhizobium meliloti*. *Appl. Environ. Microbiol.* 66:3621-3623.
- Taylor RK, Guilford PJ, Clark RG, Hale CN, Forster RLS (2001). Detection of *Erwinia amylovora* in plant material using novel polymerase chain reaction (PCR) primers. *N.Z. J. Crop Hort. Sci.* 29: 35-43.
- Theron J, Morar D, du Preez M, Brözel VS, Venter SN (2001). A sensitive seminested PCR method for the detection of *Shigella* in spiked environmental water samples. *Wat. Res.* 35:869 - 874.
- Theron J, Cilliers J, du Preez M, Brözel VS, Venter SN (2000). Detection of toxigenic *Vibrio cholerae* from environmental water samples by an enrichment broth cultivation-pit-stop semi-nested PCR procedure. *J. Appl. Microbiol.* 89:539-546.
- Vos P, Hogers R, Bleeker M, Reijans M, van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper J, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.
- Wang C, Knill E, Glick BR, Defago G (2000). Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHAO and its gacA derivative CHA96 on their growth-promoting and disease-suppressive capabilities. *Can. J. Microbiol.* 46:898-907.
- Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18:7213-7218.
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
- Yost CK, Nattress FM (2000). The use of multiplex PCR reactions to characterize populations of lactic acid bacteria associated with meat spoilage. *Lett. Appl. Microbiol.* 31:129-133.
- Zabeau M, Vos P (1993). Selective restriction fragment amplification: a general method for DNA fingerprinting. Publication 0 534 858 A1, bulletin 93/13. European Patent Office, Munich, Germany.