Journal of Food Technology 9 (1): 9-17, 2011
ISSN: 1684-8462
© Medwell Journals, 2011

Modern Trends to Investigate Food Borne Listeriosis

Rajeev Kumar
Department of Veterinary Public Health, College of Veterinary and Animal Sciences,
G.B. Pant University of Agriculture and Technology, U.S. Nagar,
Pantnagar 263145, Uttarakhand, India

Abstract: This study concerned to investigate food borne pathogens *Listeria* species. The genus *Listeria* is an opportunistic pathogen in humans and various animal species. *Listeria* sp. are wide distribution. Despite their poor survival in nutrient-deficient, unpolluted seawater and spring water, *Listeria* sp. Healthy cows can serve as reservoirs for *L. monocytogenes* and secrete the organism in milk. Almost all cases (about 98%) of human listeriosis and 85% of animal cases are due *L. monocytogenes* although, rarely *L. seeligeri* and *L. ivanovii* have also been implicated. *L. ivanovii*, the other pathogenic species of genus *Listeria* has been thought to be frequently associated with abortions in sheep and in cattle. In humans, *L. ivanovii* infection is rare and has been reported from a patient with AIDS.

Key words: Listeriosis, food-borne, disease, isolation, molecular methods, humans, animal, India

INTRODUCTION

The genus *Listeria* includes 6 species namely *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. grayi*. Of these *L. monocytogenes* is an opportunistic pathogen in humans and animal species whereas *L. ivanovii* mainly affects ruminants causing abortion only occasionally occurring in man.

The genus *Listeria* consists of a group of gram-positive bacteria of low G+C content closely related to *Bacillus*, *Enterococcus*, *Clostridium*, *Streptococcus* and *Staphylococcus* sp. *Listeria* sp. are isolated from a diversity of environmental sources including soil, water, effluents, a large variety of foods and the feces of humans and animals. The natural habitat of these bacteria is thought to be decomposing plant matter in which they live as saprophytes.

Listeria are a small, gram-positive, nonsporulating, facultatively anaerobic rod which measures 1-2×0.5 microns and shows characteristic tumbling motility at or around 25°C. Listeria are able to multiply at high salt concentrations (10% NaCl) and at a broad range of pH (pH 4.5-9) and temperature (0-45°C, optimum 30-37°C). All cases (about 98%) of human listeriosis and 85% of animal cases are due *L. monocytogenes* (McLauchlin, 1987) although, rarely *L. seeligeri* and *L. ivanovii* have also been implicated (Palmer *et al*., 1998). *L. ivanovii*, the other pathogenic species of genus *Listeria* has been thought to be frequently associated with abortions in sheep (Sergeant *et al*., 1991) and in cattle Alexander *et al*. (1992), Iijima *et al*. (2004). In humans, *L. ivanovii* infection is rare and has been reported from a patient with AIDS (Cummins *et al*., 1994). Different molecular methods have been applied to diagnose, discriminate and survey food borne pathogens. PCR-RFLP, PCR and PFGE are the most widely and successfully used.

However, these methods are unable to replace conventional and internationally standardised phenotyping. Electronic database libraries of the different genomic profiles will enable continuous surveillance of infections and detection of possible infection clusters at an early stage. Furthermore, whole-genome sequence data have opened up new insights into epidemiological surveillance. Using molecular, genetic typing methods, *L. monocytogenes* is separated into 3 lineages; lineage I is composed of serotypes of 1/2b, 3b, 4b, 4d and 4e lineage II of serotypes 1/2a, 1/2c, 3a, 3c and lineage III of serotypes 4a and 4c (Schönberg *et al*., 1996).

Listeria a food-borne pathogen: *Listeria* sp. are robust bacteria with wide distribution. Despite their poor survival in nutrient-deficient, unpolluted seawater and spring water, *Listeria* sp. readily replicate in nutrient-rich, contaminated waters, sewage, sludge, soil, foods and animal hosts. In order to lower the incidence of *Listeria* sp. in raw food materials and farm-raised animals, it is important to adapt appropriate farming and husbandry practices that lessen Listeria contamination. Healthy cows can serve as reservoirs for *L. monocytogenes* and secrete the organism in milk. Contamination of milk may also occur through accidental contact with faeces and silage.

Indeed with mortality rates on average approaching 30%, *L. monocytogenes* far exceeds other common foodborne pathogens such as *Salmonella enteritidis*

(with a mortality of 0.38%), *Campylobacter* species (0.02-0.1%) and *Vibrio* species (0.005-0.01%) in terms of disease severity (Mead *et al*., 1999). Pathogenic Listeria are able to breach endothelial and epithelial barriers of the infected hosts including the intestinal, blood-brain and fetoplacental barrier are able to invade and replicate in phagocytic and nonphagocytic cells (Mead *et al*., 1999). The genes encoding the virulence-associated proteins PIPLC, LLO, Mpl, ActA and PC-PLC are located in a 9.6 kb virulence gene cluster (Gouin *et al*., 1994) which is principally regulated by a pleiotropic virulence regulator, prfA (a 27 kDa protein encoded by prfA). In addition, to these virulence associated genes and proteins, several other genes such as *iap*, *bsh*, *vip*, *inlJ*, *auto*, *ami*, *bilA* are also involved in *L. monocytogenes* virulence and pathogenicity.

The resistance of *L. monocytogenes* to acidic conditions and to bile salts makes it particularly adept at infecting the gastrointestinal tract (Hamon *et al*., 2004).

Disease in humans: During the early stages of infection, human listeriosis often displays non-specific flu-like symptoms (e.g., chills, fatigue, headache and muscular and joint pain) and gastroenteritis. However, without appropriate antibiotic treatment it can develop into septicaemia, meningitis, encephalitis, abortion and in some cases, death (Bubert *et al*., 1999). The incubation period between exposure (consumption of contaminated foods) and onset of *listeriosis* varies between 1 day and 3 months (Linnan *et al*., 1988).

The majority of cases in adults and juveniles occur amongst the immunosuppressed, i.e., patients receiving steroid therapy or with malignant neoplasms. Other at risk groups include patients with AIDS, diabetics, elderly people and individuals with alcoholic liver disease. Approximately one-third of patients with listerial meningitis and around 10% with primary bacteraemia are immuno-competent (Cummins *et al*., 1994). Listeriosis in pregnancy manifests as severe systemic infection in the unborn or newly delivered infant as well as a mild influenza-like bacteraemic illness in the pregnant woman. Infection can occur at any stage of pregnancy (Bubert *et al*., 1999).

Disease in animals: The disease in animals is broad ranging from asymptomatic infection and carriage to uncommon cutaneous lesions or various focal infections such as conjunctivitis, urethritis, endocarditis and severe disturbance of gait followed by death. Abortions and perinatal deaths are common in cattle and sheep. *L. monocytogenes* is a well-recognized cause of mastitis, abortion, repeat breeding, infertility, encephalitis and septicaemia in cattle (Shakuntala *et al*., 2006). *L. ivanovii* has also been implicated as a cause of abortion in cattle and sheep but occurs less frequently than *L. monocytogenes*. Listeric infections and abortions usually develop in the late winter or early spring. Abortions are most commonly recognized in the last trimester of pregnancy, frequently in the absence of other clinical signs (Alexander *et al*., 1992).

Methods for confirmation/differentiation of Listeria: As *L. monocytogenes* is morphologically indistinguishable from other *Listeria* species additional, laboratory testing is required to differentiate *L. monocytogenes* from other *Listeria* species. *L. ivanovii* is differentiated biochemically from *L. monocytogenes* and other *Listeria* species by its production of a wide, clear or double zone of haemolysis on sheep or horse blood agar, strong lecithinase reaction with or without charcoal in the medium, a positive Christie-Atkins-Munch-Petersen (CAMP) reaction with *Rhodococcus equi* but not with haemolytic *Staphylococcus aureus* and fermentation of D-xylose but not L-rhamnose (Rocourt and Catimel, 1985). *L. monocytogenes* requires charcoal for its lecithinase reaction. Assay based on phosphatidylinositol specific Phospholipase C activity (PI-PLC) has been described for the discrimination of pathogenic and non-pathogenic *Listeria* species based on which haemolytic but non-pathogenic species, i.e., *L. seeligeri* can be separated from the haemolytic and pathogenic species i.e., *L. monocytogenes* and *L. ivanovii* (Notermanns *et al*., 1991). The application of a multiplex PCR assay that selectively amplifies a commonly shared region of the *iap* gene that facilitates the differentiation of all 6 *Listeria* species in a single test has been developed (Bubert *et al*., 1999).

Investigation of listeriosis: Listeriosis can be tentatively diagnosed on the basis of clinical symptoms and demonstration of the organisms in smear by Gram's staining. The organism can be isolated from clinical specimens such as blood, Cerebrospinal Fluid (CSF) and meconium of newborns or foetus in abortion cases and faeces, vomitus, food stuffs/animal feed and vaginal secretions of infected individuals or animals.

Isolation of Listeria: It has been challenging to isolate Listeria from food or other samples and this explains why it remained unnoticed as a major food pathogen until recently. The earliest method available was the cold enrichment technique (Gray, 1948) which remained the only available method for many years (Fig. 1).

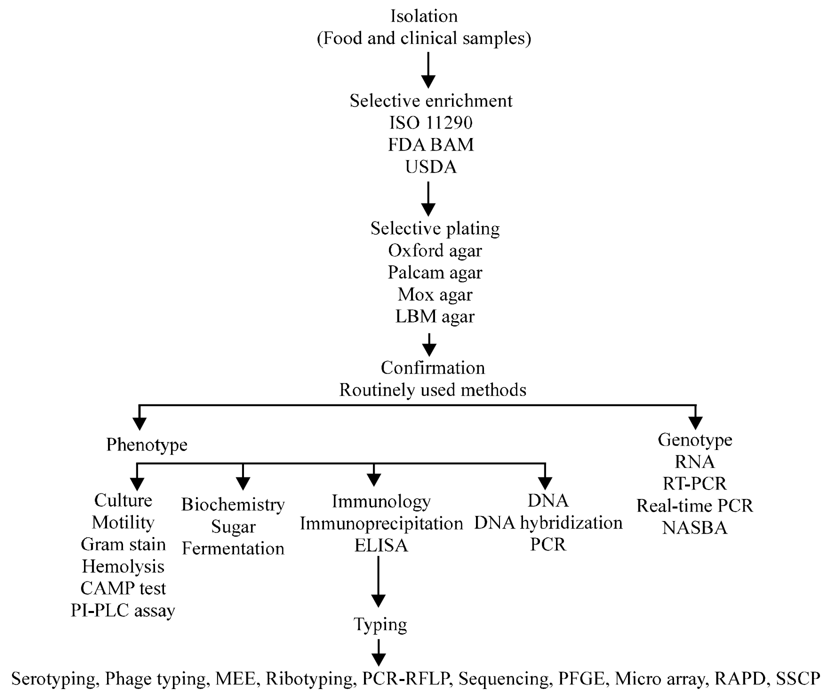

Fig. 1: Method for the isolation of Listeria

Scheme for isolation, identification and typing methods for Listeria and *L. monocytogenes* in foods, environmental and clinical samples: Owing to many food borne outbreaks of listeriosis, a zero tolerance level (absence in 25 g of food) has been implemented. Therefore, the tests must be able to detect one Listeria organism per 25 g of food if to be approved by the regulatory agencies. Two of the most widely used culture reference methods for detection of Listeria in all foods are the FDA Bacteriological and Analytical Method (BAM) and the International Organization of Standards (ISO) 11290 method.

The FDA method was designed for processing dairy products whereas the USDA method was designed and has been officially recommended primarily for meat and poultry products (Brackett and Beuchat, 1989) the latter being slightly superior for detection of *L. monocytogenes* in foods and environmental samples. In FDA, BAM method, sample (25 g) is enriched for 48 h at 30°C in Listeria Enrichment Broth (LEB) (Lovette *et al*., 1987) containing the selective agents acriflavin, nalidixic acid and the antifungal agent cycloheximide followed by plating onto selective agar (Oxford, PALCAM, MOX or LPM) (Cummins *et al*., 1994). The ISO 11290 method employs a two stage enrichment process: the first enrichment in half Fraser broth for 24 h (Fraser and Sperber, 1988) then an aliquot is transferred to full strength Fraser broth for further enrichment followed by plating on Oxford and PALCAM agars. Fraser broth also contains the selective agents acriflavin and nalidixic acid as well as esculin which allows detection of β-D-glucosidase activity by Listeria observed as blackening of the growth medium (Gasanov *et al*., 2005). The USDA and Association of Analytical Chemists (AOAC/IDF) methods use a modification of University of Vermont Medium (UVM) (Donnelly and Baigent, 1986), containing acriflavin and nalidixic acid for primary enrichment, followed by secondary enrichment in Fraser broth and plating onto Modified Oxford (MOX) agar (Gasanov *et al*., 2005). Recent improvements to traditional methods include improved isolation media, alternative plating techniques, rapid and automated pathogen monitoring and identification systems. Examples include VITEK (BioMerieux, Marcy-Etoile, France) and the MicroLog^{TM} system (BiOLOG, CA, USA). Novel methods to measure bacterial growth have included impedance measurements and ATP bioluminescence for bacterial detection in food (Cummins *et al*., 1994; Gracias and McKillip, 2004).

Chromogenic media: Phosphotidylinositol-specific phospholipase C is an enzyme produced only by *L. monocytogenes* and *L. ivanovii* which hydrolyses a specific substrate added to the medium, producing an opaque halo around the colonies. The chromogenic media those are commercially available include Agar

Listeria according to Ottoviani and Agosti, BCM *L. monocytogenes* detection system, CHROM agar and Rapid *L. mono*. Chromogenic media are simple, cost effective and easy to interpret (Gasanov *et al*., 2005).

Virulence determination: *L. monocytogenes* strains are highly pathogenic others are relatively avirulent and cause little harm in the host. A variety of methods have been developed to gauge the virulence of *L. monocytogenes* strains. Besides, haemolysin, mouse pathogenicity, tissue culture systems and the detection of virulence associated proteins and genes have been used to identify virulent Listeria.

Immunological tests

Conventional tests: Serological methods have been reported to be non-specific because of antigenic cross-reactivity between *L. monocytogenes* and other Gram-positive bacteria such as *Staphylococci*, *Enterococci* and *Bacillus* sp. (Gray and Killinger, 1966).

Enzyme Linked Immunosorbent Assay (ELISA): ELISAs for *L. monocytogenes* detection are either based on polyclonal antibody or on monoclonal Antibodies (mABs). There are a number of ELISA formats including direct ELISAs, sandwich ELISAs and competitive ELISAs. mAbs based ELISA and dot-blot assays have been reported to identify *Listeria*. sp. in food (Farber and Speirs, 1987) and clinical samples (McLauchlin *et al*., 1988).

MATERIALS AND METHODS

Molecular methods

DNA probes: The presence of a target sequence is detected using a single-stranded nucleic acid that is enzyme or radiolabeled (Datta *et al*., 1988), reported the first DNA probe wherein a Hind III-Hind II fragment of a presumptive haemolysin gene was used in a trial for specific detection of *L. monocytogenes*. Different DNA probes such as digoxigenin-labelled synthetic oligonucleotide probe, encoding part of *listeriolysin* gene on a 16S rRNA sequence have been developed. DNA probes targeting the inlA and plcA and the prfA genes have also been developed (Datta *et al*., 1988).

Nucleic acid amplification: The first reported PCR for identification of *L. monocytogenes*, the hly sequence published were used (Mengaud *et al*., 1988). This PCR was used to detect *L. monocytogenes* in water whole milk and human cerebrospinol fluid. Standard PCR followed by dot-blot hybridization has come out as an encouraging approach for the diagnosis of *Listeria meningitis* (Jaton *et al*., 1992).

Reverse Transcription (RT)-PCR: Testing of food or environmental samples for pathogenic Listeria should only target living organisms since only live Listeria cells can cause disease. The choice of RNA or mRNA as a target for food pathogen testing has gathered increasing favour since the presence of mRNA is an indication of the living state of the cell (Keer and Birch, 2003; Novak and Juneja, 2001).

Real-time PCR: Real time PCR has been used to identify and quantify *L. monocytogenes* in foods and clinical samples in several studies (Hein *et al*., 2001; Hough *et al*., 2002). Real-time PCR is quantitative which is a significant advantage over other molecular methods and so this technology is extremely attractive for food testing and epidemiological investigations.

Other methods: Immunomagnetic separation used primarily to isolate strains of *L. monocytogenes* from pure cultures as well as from heterogenous suspensions has been viewed as a new approach for extraction and isolation of pathogenic bacteria directly from foods (Skjerve *et al*., 1990).

Methods for subtyping of listeria: The availability of subtyping procedures to track individual strains involved in listeriosis outbreaks and to examine the epidemiology and population genetics of *L. monocytogenes* bacteria is integral to control and prevention programs aimed at limiting listeriosis.

Application of subtyping methods also provides insight into the population genetics, epidemiology, ecology and evolution of *L. monocytogenes*. A variety of conventional, phenotypic and DNA-based subtyping methods have been described for differentiation of *L. monocytogenes* beyond the species and subspecies levels (Graves *et al*., 1999). While, DNA-based subtyping methods are generally more discriminatory and amenable to inter laboratory standardization and are thus increasingly replacing phenotype-based subtyping methods. Commonly used phenotype-based subtyping methods for *L. monocytogenes* and other food-borne pathogens include serotyping (Palumbo *et al*., 2003), phage typing (Audurier *et al*., 1984), Esterase typing (Graves *et al*., 1999) and Multilocus Enzyme Electrophoresis (MLEE) (Audurier *et al*., 1984).

The genetic subtyping approach encompasses PCR based approaches (e.g., random amplified polymorphic DNA and amplified fragment length polymorphism) (Lawrence and Gilmou, 1995), PCR-restriction fragment length polymorphism (PCR-RFLP) (Paillard *et al*., 2003), ribotyping (Manfreda *et al*., 2005), pulsed-field gel

electrophoresis (Aarts *et al.*, 1999; Jeffers *et al.*, 2001), AFLP (Fonnesbech *et al.*, 2004) and DNA sequencing-based subtyping techniques, e.g., Multilocus Sequence Typing (MLST) (Graves and Swaminathan, 2001; Bruce *et al.*, 1995) and DNA microarray (Borucki *et al.*, 2003).

The phenotypic subtyping approach occasionally suffers from low discrimination and reproducibility, the genetic subtyping approach is highly sensitive, discriminatory and reproducible. For improved subtyping discrimination, a combination of two or more subtyping techniques be they gene or phenotype based is often used in practice for epidemiologic investigation of *L. monocytogenes* outbreaks.

Pulsed-Field Gel Electrophoresis (PFGE): In PFGE, restriction patterns of whole bacterial genomes are analyzed and compared. The bacterial chromosome is digested by a selected rare cutting enzyme to yield a moderate number of DNA fragments. To protect the chromosomal DNA from mechanical breakage, the bacteria are immobilised by mixing the bacterial suspension with melted agarose before the cells are lysed. The blocks containing purified and digested DNA are loaded onto agarose gels and the DNA fragments are separated by pulsed-field electrophoresis in which the orientation of the electrical field alternates. PFGE used with various restriction enzymes has been considered the golden method in the subtyping of bacteria. Although, all strains can normally in contrast to phenotypic methods be subtyped by PFGE, this method alone is unable to replace conventional and internationally standardized phenotypic methods. A particular disadvantage of the PFGE method has previously been that it is time consuming and labour intensive. However, a new shorter protocol has been described that can complete the subtyping process within 30 h (Graves and Swaminathan, 2001).

This makes PFGE more competitive in relation to other genotyping methods. PFGE has already been successfully used in short-term epidemiological investigations for many food-borne bacterial pathogens. It is a very accurate and reproducible method. Comparison of profiles with usually only 10-20 bands is relatively easy and computer-based analysis with the possibility of creating database libraries is available. These facts have made it the most commonly used method also for long-term surveillance.

Electronic database libraries of genomic profiles: Electronic database libraries of the different PFGE profiles of human *Salmonella enterica*, *L. monocytogenes* and STEC strains have been created in different countries. In these libraries, the PFGE profiles can be compared with each other much more quickly than by just the naked eye. This helps in more easily detecting possible infection clusters at an early stage of the outbreak. A similar electronic database (Pulse Net) of the PFGE profiles of various bacterial strains has been in use in the USA where it has demonstrated its value in the early recognition of outbreaks and rapid identification of their sources.

RESULTS AND DISCUSSION

Genomics in diagnostics: The availability of genome sequences of *L. monocytogenes* serovars, *L. innocua* and *L. welshimeri* (Glaser *et al.*, 2001; Hain *et al.*, 2006; Nelson *et al.*, 2004) have provided insight into the molecular basis of the pathogenesis determinants of *Listeria* species. Further, sequencing of the strains comprising other species of this genus will provide a rich resource for understanding the sources of variation and evolutionary history.

Emerging diagnostic technologies

MALDI: Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOFMS) has become a valuable tool for the analysis of microorganisms (Krishnamurthy *et al.*, 1996). The accuracy and speed with which data can be obtained by MALDI-TOF-MS make this a potentially important tool for biological public health hazards, food processing, blood screening and disease diagnoses. The MALDI technique couples high sensitivity with accuracy and is ideal for the detection of high and low molecular weight proteins. MALDI-TOF MS has the potential for rapidly distinguishing between pathogenic and nonpathogenic species of bacteria (Krishnamurthy *et al.*, 1996; Krishnamurthy and Ross 1997). In addition, mass spectra, obtained from unknown bacterial samples can be used for comparison with reference libraries of known organisms for a correct identification. This concept using a reference database with which unknown bacterial samples can be identified on the basis of comparisons with reference spectra, currently is the most promising approach for microorganism identification based on MALDI-TOF MS.

The goal is the elucidation of a reproducible genus species and strain-specific fingerprint for any given organism (Krishnamurthy *et al.*, 1996).

A rapid method involving Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) have been developed that showed promise for identification of *Listeria* species and typing and even allowed for differentiation at the level of clonal lineages among pathogenic strains of *L. monocytogenes*

(Barbuddhe *et al*., 2008). A total of 146 strains of different *Listeria* species and serotypes as well as clinical isolates were analyzed. The method was compared with the PFGE analysis of 48 Listeria strains comprising *L. monocytogenes* isolated from foodborne epidemics and sporadic cases, isolates representing different serotypes as well as a number of Listeria strains whose genomes have been completely sequenced. MALDI-TOF MS technique examined the chemistry of major proteins, yielding profile spectra consisting of a series of peaks, a characteristic fingerprint mainly derived from ribosomal proteins.

Specimens could be prepared in a few minutes from plate or liquid cultures and a spectrum could be obtained within 1 min. Mass spectra derived from Listeria isolates showed characteristic peaks, conserved at both species and lineage-level. MALDI-TOF MS fingerprinting may have potential for Listeria identification and subtyping and may improve infection control measures (Barbuddhe *et al*., 2008).

Biosensors: Diagnostic biosensors are a group of devices and technologies that use a biologically derived material immobilized on a detection platform to measure the presence of one or more analytes (Ivnitski *et al*., 2000). For applications in food microbiology analysis, an ideal biosensor would be a self-contained, automated system capable of pathogen detection directly from a food matrix without pre-enrichment and also be capable of differentiating live from dead cells (Ivnitski *et al*., 2000). To date, biosensor technologies developed for the specific detection of pathogenic bacteria in food samples have included metabolism-based, antibody-based and DNA-based systems.

A thorough review of biosensor technology for environmental pollutants and food contaminants is provided and proved (Baeumner, 2003) covering assay formats and target recognition elements including nucleic acids, enzymes, antibody, whole-cell and bio-electric biosensors. Nucleic-acid-based biosensors that have found application in the food industry include Quartz Crystal Microbalances (QCM) and optical detection systems.

The current generation of optical biosensors use Surface Plasmon Resonance (SPR) to monitor biomolecular interactions on a surface in real time (Rand *et al*., 2002). An advantage of SPR-based systems is that no labelling of target molecule is required. Targets have included whole-cell detection of *L. monocytogenes* (Leonard *et al*., 2004). The applications of this technology for food borne pathogen identification have yet to be fully realized (Mannelli *et al*., 2005; Wang *et al*., 2004).

Microarrays: DNA microarrays consist of large numbers of probes (either oligonucleotides or cDNAs) immobilized on a solid surface such as specially treated glass. The first practical demonstrations of microarrays were treated glass slides with probes deposited in spots onto the surface. Hybridizations are performed by application of labeled nucleic acid target in a liquid state to the microarray surface. Following appropriate hybridization and washing steps, target nucleic acid bound to probes on the array surface are visualized using a microarray scanner. Arrays that discriminate closely related strains and recognize genetic markers can be constructed and they could help us understand the epidemiology and potential pathogenic characteristics of infecting microbial strains. Microarray-based diagnostics platforms reported in the literature are typically in the form of proof of principle of these technologies using either PCR products or synthetic oligonucleotides to demonstrate both sensitivity and specificity (Bodrossy and Sessitsch, 2004). A microarray based assay incorporating signal amplification and suspension microarray technologies has been reported for the identification and subtyping of *L. monocytogenes* from genomic DNA (Borucki *et al*., 2005). The developments in biosensor and microarray technologies have generated promising results to date. However, for application in bacterial food safety, several important issues remain.

CONCLUSION

Several molecular genetic methods have been applied to diagnose, discriminate and survey food borne pathogens. PCR, PCR-RFLP and PFGE are the most widely and successfully used. However, these methods are unable to replace conventional and internationally standardised phenotyping. Electronic database libraries of the different genomic profiles will enable continuous surveillance of infections and detection of possible infection clusters at an early stage. Furthermore, whole-genome sequence data have opened up new insights into epidemiological surveillance. Molecular methods were DNA probes, Real-Time PCR, Reverse Transcription (RT)-PCR, Nucleic Acid amplification, Biosensors and other molecular typing technique most efficient in recent time.

REFERENCES

Aarts, H.J., L.E. Hakemulder and M. van Hoef, 1999. Genomic typing of *Listeria monocytogenes* strains by automated laser fluorescence analysis of amplified fragment length polymorphism fingerprint patterns. Int. J. Food Microbiol., 49: 95-102.

Alexander, A.V., R.L. Walker, B.J. Johnson, B.R. Charlton and L.W. Woods, 1992. Bovine abortions attributable to Listeria ivanovii: Four cases (1988-1990). J. Am. Vet. Med. Asso., 200: 711-714.

Audurier, A., A.G. Taylor, B. Carbonnelle and J.A. McLauchlin, 1984. Phage typing system for *Listeria monocytogenes* and Its use in epidemiological studies. Clin. Invest. Med., 7: 229-232.

Baeumner, A.J., 2003. Biosensors for environmental pollutants and food contaminants. Anal. Bioanal. Chem., 377: 434-445.

Barbuddhe, S.B., T. Maier, G. Schwarz, M. Kostrzewa and H. Hof *et al*., 2008. Rapid identification and typing of *Listeria* species using matrix assisted laser desorption ionization-time of flight mass spectrometry. Applied Environ. Microbiol., 74: 5402-5407.

Bodrossy, L. and A. Sessitsch, 2004. Oligonucleotide microarrays in microbial diagnostics. Curr. Opin. Microbiol., 7: 245-254.

Borucki, M.K., J. Reynolds, D.R. Call, T.J. Ward, B. Page and J. Kadushin, 2005. Suspension microarray with dendrimer signal amplification allows direct and high-throughput subtyping of *Listeria monocytogenes* from genomic DNA. J. Clin. Microbiol., 43: 3255-3259.

Borucki, M.K., M.J. Krugb, W.T. Muraokab and D.R. Call, 2003. Discrimination among *Listeria monocytogenes* isolates using a mixed genome DNA microarray. Vet. Microbiol., 92: 351-362.

Brackett, R.E. and L.R. Beuchat, 1989. Methods and media for the isolation and cultivation of *Listeria monocytogenes* from various foods. Int. J. Food Microbiol., 8: 219-223.

Bruce, J.L., R.J. Hubner, E.M. Cole, C.I. McDowell and J.A. Webster, 1995. Sets of EcoRI fragments containing ribosomal RNA sequences are conserved among different strains of *Listeria monocytogenes*. Proc. Natl. Acad. Sci., 92: 5229-5234.

Bubert, A., Z. Sokolovic, S.K. Chun, L. Papatheodorou, A. Simm and W. Goebel, 1999. Differential expression of *Listeria monocytogenes* virulence genes in mammalian host cells. Mol. Gen. Genet., 261: 323-326.

Cummins, A.I., A.K. Fielding and J. McLauchlin, 1994. *Listeria ivanovii* infection in a patient with AIDS. J. Infect., 28: 89-91.

Datta, A.R., B.A. Wentz and W.E. Hill, 1988. Identification and enumeration of beta-hemolytic *Listeria monocytogenes* in naturally contaminated dairy products. J. Assoc. Off. Anal. Chem., 71: 673-675.

Donnelly, C.W. and G.J. Baigent, 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Applied Environ. Microbiol., 52: 689-694.

Farber, J.M. and J.I. Speirs, 1987. Monoclonal antibodies directed against the flagellar antigens of *Listeria* spp. and their potential in EIA based methods. J. Food Prot., 50: 479-484.

Fonnesbech, V.B., V. Fussing, B. Ojeniyi, L. Gram and P. Ahrens, 2004. High-resolution genotyping of *Listeria monocytogenes* by fluorescent amplified fragment length polymorphism analysis compared to pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, ribotyping and PCR-restriction fragment length polymorphism analysis. J. Food Prot., 67: 1656-1665.

Fraser, J.A. and W.H. Sperber, 1988. Rapid detection of *Listeria* spp. in food and environmental samples by esculin hydrolysis. J. Food Prot., 51: 762-765.

Gasanov, U., D. Hughes and P.M. Hansbro, 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: A review. FEMS Microbiol. Rev., 29: 851-875.

Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok and A. Amend *et al*., 2001. Comparative genomics of *Listeria* species. Science, 294: 849-852.

Gouin, E., J. Mengaud and P. Cossart, 1994. The virulence gene cluster of *Listeria monocytogenes* is also present in *Listeria ivanovii*, an animal pathogen and *Listeria seeligeri*, a nonpathogenic species. Infect. Immun., 62: 3550-3553.

Gracias, K.S. and J.L. McKillip, 2004. A review of conventional detection and enumeration methods for pathogenic bacteria in foods. Can. J. Microbiol., 50: 883-890.

Graves, L.M. and B. Swaminathan, 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int. J. Food Microbiol., 65: 55-62.

Graves, L.M., B. Swaminathan and S.B. Hunter, 1999. Subtyping *Listeria Monocytogenes*. In: *Listeria*, *Listeriosis* and Food Safety, Ryser, E. and E. Marth (Eds.). Marcel Dekker, New York.

Gray, M.L. and A.H. Killinger, 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev., 30: 309-382.

Gray, M.L., 1948. A new technique for isolating listerelle from the bovine brain. J. Bacteriol., 55: 471-476.

Hain, T., C. Steinweg, C.T. Kuenne, A. Billion and R. Ghai *et al*., 2006. Whole-genome sequence of *Listeria welshimeri* reveals common steps in genome reduction with *Listeria innocua* as compared to *Listeria monocytogenes*. J. Bacteriol., 188: 7405-7415.

Hamon, M, H. Bierne and P. Cossart, 2004. *Listeria monocytogenes*: A multifaceted model. Nat. Rev. Microbiol., 4: 423-434.

Hein, I., D. Klein, A. Lehner, A. Bubert, E. Brandl and M. Wagner, 2001. Detection and quantification of the iap gene of *Listeria monocytogenes* and *Listeria innocua* by a new real-time quantitative PCR assay. Res. Microbiol., 152: 37-46.

Hough, A.J., S.A. Harbison, M.G. Savill, L.D. Melton and G. Fletcher, 2002. Rapid enumeration of *Listeria monocytogenes* in artificially contaminated cabbage using real-time polymerase chain reaction. J. Food Prot., 65: 1329-1332.

Iijima, Y., N.T. Asako, M. Aihara and K. Hayashi, 2004. Improvement in the detection rate of diarrhoeagenic bacteria in human stool specimens by a rapid real-time PCR assay. J. Med. Microbiol., 53: 617-622.

Ivnitski, D., I. Abdel-Hamid, P. Atanasov, E. Wilkins and S. Stricker, 2000. Application of electrochemical biosensors for detection of food pathogenic bacteria. Electroanalysis, 12: 317-325.

Jaton, K., R. Sahli and J. Bille, 1992. Development of polymerase chain reaction assays for detection of *Listeria monocytogenes* in clinical cerebrospinal fluid samples. J. Clin. Microbiol., 30: 1931-1936.

Jeffers, G.T., J.L. Bruce, P.L. McDonough, J. Scarlett, K.J. Boor and M. Wiedmann, 2001. Comparative genetic characterization of *Listeria monocytogenes* isolates from human and animal *Listeriosis* cases. Microbiology, 147: 1095-1104.

Keer, J.T. and L. Birch, 2003. Molecular methods for the assessment of bacterial viability. J. Microbiol. Methods, 53: 175-183.

Krishnamurthy, T. and P.L. Ross, 1997. Rapid identification of bacteria by direct matrix assisted laser desorption/ionization mass spectrometric analysis of whole cells. Rapid Commun. Mass Spectrom., 10: 1992-1996.

Krishnamurthy, T., P.L. Ross and U. Rajamani, 1996. Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom., 10: 883-888.

Lawrence, L.M. and A. Gilmou, 1995. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Applied Environ. Microbiol., 61: 2139-2144.

Leonard, P., S. Hearty, J. Quinn and R. O'Kennedy, 2004. Generic approach for the detection of whole *Listeria monocytogenes* cells in contaminated samples using surface plasmon resonance. Biosensors Bioelectronics, 19: 1331-1335.

Linnan, M.J., L. Mascola, X.D. Lou, V. Goulet and S. May *et al*., 1988. Epidemic *Listeriosis* associated with Mexican-style cheese. N. Engl. J. Med., 319: 823-828.

Lovette, J., D.W. Frances and J.M. Hunt, 1987. *Listeria monocytogenes* in raw milk: Detection, Incidence and pathogenicity. J. Food Prot., 50: 188-192.

Manfreda, G., A. De Cesare, S. Stella, M. Cozzi and C. Cantoni, 2005. Occurrence and ribotypes of *Listeria monocytogenes* in Gorgonzola cheeses. Int. J. Food Microbiol., 102: 287-293.

Mannelli, I., M. Minunnia, S. Tombellia, R. Wang, M.M. Spiritia and M. Mascinia, 2005. Direct immobilisation of DNA probes for the development of affinity biosensors. Bioelectrochemistry, 66: 129-138.

McLauchlin, J., 1987. *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in human. J. Applied Bacteriol., 63: 1-11.

McLauchlin, J., A. Black, H.T. Green, J.Q. Nash and A.G. Taylor, 1988. Monoclonal antibodies show *Listeria monocytogenes* in necropsy tissue samples. J. Clin. Pathol., 41: 983-988.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig and J.S. Bresee *et al*., 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

Mengaud, J., M.F. Vicente, J. Chenevert, J.M. Pereira and C. Geoffroy *et al*., 1988. Expression in *Escherichia coli* and sequence analysis of the listeriolysin O determinant of *Listeria monocytogenes*. Infect. Immun., 56: 766-771.

Nelson, K.E., D.E. Fouts, E.F. Mongodin, J. Ravel and R.T. DeBoy *et al*., 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. Nucleic Acids Res., 32: 2386-2392.

Notermanns, S.H., J. Dufrenne and M. Leimeister-Wachter, 1991. Phosphatidylinositol-specific phospholipase C activity as a marker to distinguish between pathogenic and nonpathogenic *Listeria* species. Applied Environ. Microbiol., 57: 2666-2673.

Novak, J.S. and V.K. Juneja, 2001. Detection of heat injury in *Listeria monocytogenes* Scott A. J. Food Prot., 64: 1739-1743.

Paillard, D., V. Dubois, R. Duran, F. Nathier, C. Guittet, P. Caumette and C. Quentin, 2003. Rapid identification of *Listeria* species by using restriction fragment length polymorphism of PCR-amplified 23S rRNA gene fragments. Applied Environ. Microbiol., 69: 6386-6392.

Palmer, S.R., E.J.L. Soulsby and D.I.H. Simpson, 1998. Zoonoses (Biology, Clinical Practice and Public Health Control). Oxford University Press Inc., New York.

Palumbo, J.D., M.K. Borucki, R.E. Mandrell and L. Gorski, 2003. Serotyping of *Listeria monocytogenes* by enzyme-linked immunosorbent assay and identification of mixed-serotype cultures by colony immunoblotting. J. Clin. Microbiol., 41: 564-571.

Rand, A.G., Y. Ye, C.W. Brown and S.V. Letcher, 2002. Optical biosensors for food pathogen detection. Food Technol., 56: 32-39.

Rocourt, J. and B. Catimel, 1985. Biochemical characterization of species in the genus Listeria. Zentralbl. Bakteriol. Mikrobiol. Hyg., 260: 221-231.

Schönberg, A., E. Bannerman, A.L. Courtieu, R. Kiss, J. McLauchlin, S. Shah and D. Wilhelms, 1996. Serotyping of 80 strains from the WHO multicentre international typing study of *Listeria monocytogenes*. Int. J. Food Microbiol., 32: 279-287.

Sergeant, E.S.G., S.C.J. Love and A. McInnes, 1991. Abortions in sheep due to *Listeria ivanovii*. Aust. Vet. J., 68: 39-43.

Shakuntala, I., S.V.S. Malik, S.B. Barbuddhe and D.B. Rawool, 2006. Isolation of *Listeria monocytogenes* from buffaloes with reproductive disorders and its confirmation by polymerase chain reaction. Vet. Microbiol., 117: 229-234.

Skjerve, E., L.M. Rorvik and O. Olsvik, 1990. Detection of *Listeria monocytogenes* in foods by immunomagnetic separation. Applied Environ. Microbiol., 56: 3478-3481.

Wang, R., S. Tombelli, M. Minunni, M.M. Spiriti and M. Mascini, 2004. Immobilisation of DNA probes for the development of SPR-based sensing. Biosensors Bioelectronics, 20: 967-974.