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Advances in Proteomics-based Detection Techniques of *Listeria monocytogenes*: a Potential Risk in New Zealand

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Abstract

Listeria monocytogenes is a Gram-positive foodborne pathogen, which is the causative agent of listeriosis. Ninety percent of listeriosis cases in New Zealand are associated with the consumption of food. Proteomics is the large-scale study of proteins based on their structures and functions. Proteomic mapping of food-borne pathogens has demonstrated its potential to be used as a rapid and effective detection tool. This article discusses the seriousness of L. monocytogenes illnesses in New Zealand and proteomics studies undertaken to detect and characterise this food-borne pathogen.

Key words: Listeris monocytogenes, food-borne pathogen, proteomic analysis

Introduction

Listeria monocytogenes is a Gram-positive, facultative anaerobic, rod-shaped bacterium. The genus Listeria is classified in the family *Listeriaceae* (previously known as *Corynebacteriaceae*) with microbiological features as non-spore formers, catalase-positive, and oxidase-negative. It expresses a beta haemolysin that causes the destruction of red blood cells. Listeria usually infects humans through contaminated vegetables or unpasteurized dairy products. One of the unique features of this bacterium is its tumbling motility when viewed with light microscopy. This bacterium is actively motile by means of peritrichous flagella at room temperature (20−25°C), but the synthesis of the flagella does not occur at body temperature (37°C). When present, the flagella cause the bacterial cells to move within eukaryotic cells by explosive polymerization of actin filaments (Hain et al. 2006; de Velde et al. 2009). The actin polymerization plays key role in infectious cycle of the bacterium.

Seriousness of Listeriosis

L. monocytogenes is the causative agent of listeriosis, which is one of the most serious food-borne illnesses worldwide. Twenty to thirty percent of clinical infections of listeriosis result in death (Mead et al. 1999; Orsi et al. 2011). This fatality rate from listeriosis is even greater than *Salmonella* and *Clostridium botulinum*. *L. monocytogenes* is distributed widely in the environment. Raw, cooked and processed foods can be a mode for the transmission of infection. *L. monocytogenes* has the ability to survive very well under freezing and other adverse growth conditions, e.g. high salt concentration, a wide range of pH and temperature and low water availability by forming biofilms. The most dangerous feature of *L. monocytogenes* is that it can grow at low temperatures (Table 1).

Table 1. Key characteristics of *Listeria monocytogenes* as a foodborne pathogen.

- Widely present in nature
- Can grow at low temperatures
- Survives very well in unfavourable environments (freezing, high salt concentration, wide range of pH and temperature)
- Ability to resist sanitation
- Forms biofilms

Outbreaks in New Zealand

In New Zealand, around 90% of cases of listeriosis are associated with the consumption of food contaminated by *L. monocytogenes* (MAF 2011). The majority of listeriosis victims are the very young, the elderly, people with the lowered immunity (usually due to chronic diseases) and pregnant women. Keeping these facts in mind, incidences of listeriosis in New Zealand will surge in the future due to the ageing population (Barnao 2011). The second contributor toward the high risk of listeriosis in New

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Zealand is the growing trend to the use of chilled ready-to-eat food (RTE) with extended shelf life (Gilbert et al. 2009). In the past, most of the outbreaks of listeriosis in New Zealand were associated with seafood, raw fish and shellfish, mussels, and ready-to-eat cooked meats. Recently, reported contaminated foods are much more diverse, such as non-dairy based flavoured dips, hummus and tahini, pate, pre-packaged salads, cooked and smoked chicken, chicken sandwiches, yoghurt, savoury dairy-based chilled dips, ice-cream and cheese, mussels, smoked fish, sliced deli meat and other small goods (Gilbert et al. 2009; MAF 2011; Crerar et al. 2011). More than 30% of reported food-borne listeriosis patients in New Zealand in 2010 did not survive (Barnao 2011). This is alarming situation which demands strict monitoring of high risk foods and improvements in detection tools.

Common Detection Methods

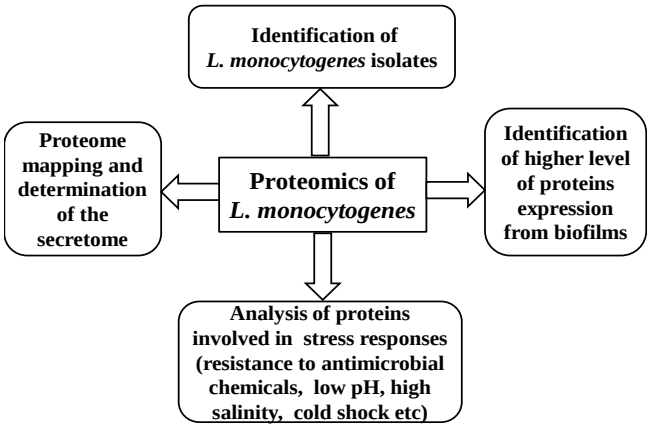
There are variety existing techniques based on colony morphology and the genetics of microorganisms to detect food-borne pathogens, including *L. monocytogenes* (Gasanov et al. 2005). The traditional methods for identification of *L. monocytogens* involved culture methods based on using selective enrichment and plating followed by the characterisation of *Listeria* spp, such as colony morphology, sugar fermentation and haemolytic properties. These methods are usually time consuming, costly, laborious and give variable results. This is mainly due to the abilities of the bacteria to change their biochemical and antigenic properties under different environmental conditions.

Over last decade, DNA-based methods were developed and now widely used. These methods are more sensitive, reliable and have better reproducibility than previous methods. The most commonly used techniques are polymerase chain reaction (PCR), microarrays, nucleic acid sequence-based amplification (NASBA) and ligation detection reaction-universal arrays (LDR-UA) (Gebretsadik et al. 2011; Mandal et al. 2011). There are some limitations with these DNA-based methods. For example, these molecular techniques only detect the specific pathogen. Analysing multiple pathogens simultaneously makes the procedures complex and expensive for the food industry. Thus, there is a need to develop faster, more sensitive and cost effective techniques for food pathogen detection, especially *L. monocytogenes*.

Proteomics analysis of *Listeria monocytogenes*

Recently, several studies attempted to use proteomics techniques to detect food-borne pathogens (Ramnath et al. 2003), especially *L. monocytogenes* (Table 2). Two-dimensional (2D) polyacrylamide gel electrophoresis is the most commonly used technique to study protein changes in *L. monocytogenes* in response to stresses such as resistance to antimicrobial chemicals, low pH, high salinity or cold shock (Duché et al. 2002; Folio et al. 2004; Gardan et al. 2003; Wemekamp-Kamphuis et al. 2004; Trost et al. 2005; Cabrita et al. 2010; Cacace et al. 2010; Rabilloud et al. 2010). With the development of quantitative proteomics, high-throughput gel- and non-gel-based protein fractionation techniques coupled with protein identification by high-throughput tandem mass spectrometry (MS/MS)-based automated software algorithms are now widely used to analyze protein expression in *L. monocytogenes*. For example, Hefford et al. (2005) identified higher levels of proteins expression in biofilms of *L. monocytogenes* using 2D analysis combined with the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) and MS/MS. Development of intracellular and extracellular proteome maps of *L. monocytogenes* has been attempted using the multiplexing fluorescent two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) and MALDI-TOF (Van den Bergh and Arckens 2004; Dumas et al. 2008; de Velde et al. 2009). More recent investigations used advanced proteome analysis techniques (two-dimensional nanoliquid chromatography coupled to ion-trap mass spectrometry (2DnLC-MS/MS) and multidimensional protein identification technology (MuDPIT) to study *L. momcytogenes* protein expression (Pucciarelli et al. 2005; Calvo et al. 2005; Donaldson et al., 2009). Most of these studies primarily focused on the identification of *L. monocytogenes*, analysis of cell wall and secretory proteins, development of a partial proteome reference map, bacterial virulence or bacterial protein expression under strict growth condition (Figure 1).

Fig. 1. Different applications of proteomics analysis to characterise *Listeria monocytogenes.*



Current research on proteomics of *L. monocytogenes* provides a promising future to develop detection technologies based on specific protein markers.

Table 2. Important publications on *Listeria monocytogenes* using proteomics analysis.

Year	Authors	Title	Technique(s) used
2003	Ramnath et al.	Development of a *Listeria monocytogenes* EGDe partial proteome reference map and comparision with the protein profiles of food isolates	Two-dimensional (2D) electrophoresis
2005	Hefford et al.	Proteomic and microscopic analysis of biofilms formed by *Listeria monocytogenes* 568	2D electrophoresis Matrix assisted laser desorption/ionization time of flight (MALDI-TOF) and mass spectrometry/mass spectrometry (MS/MS) analysis
2005	Calvo et al.	Analysis of the *Listeria* cell wall proteome by two-dimensional nanoliquid chromatography coupled to mass spectrometry	Two-dimensional nanoliquid chromatography coupled to ion-trap mass spectrometry (2DnLC-MS/MS) analysis
2005	Trost et al.	Comparative proteome analysis of secretory proteins from pathogenic and nonpathogenic *Listeria* species	2D electrophoresis MALDI-TOF/MS analysis High performance liquid chromatography (HPLC)/electrospray ionization-mass spectrometry (EI-MS)
2005	Pucciarelli et al.	Identification of substrates of the *Listeria monocytogenes* sortases A and B by a non-gel proteomic analysis	2DnLC-MS/MS analysis
2008	Dumas et al.	Comparative analysis of extracellular and intracellular proteomes of *Listeria monocytogenes* strains reveals a correlation between protein expression and serovar	2D electrophoresis MALDI-TOF/MS analysis
2009	Donaldson et al.	Comparative proteomic analysis of *Listeria monocytogenes* strains F2365 and EGD	Multidimensional protein identification technology (MuDPIT)
2009	de Velde et al.	Isolation and 2-D-DIGE proteomic analysis of intracellular and extracellular forms of *Listeria monocytogenes*	2D-DIGE MALDI-TOF analysis
2010	Cabrita et al.	A secretome-based methodology may provide a better characterization of the virulence of *Listeria monocytogenes*: preliminary results	SDS-PAGE MALDI-TOF-MS analysis
2010	Cacace et al.	Proteomics for the elucidation of cold adaptation mechanisms in *Listeria monocytogenes*	2DE electrophoresis MALDI-TOF analysis

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