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Bead array for *Listeria monocytogenes* detection using specific monoclonal antibodies

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ABSTRACT

To develop a detection method for human pathogenic *Listeria monocytogenes*, novel specific antibodies were obtained from hybridoma libraries generated by using formalin-killed and heat-killed *L. monocytogenes* as immunogens. Several monoclonal antibodies found to be specific to *Listeria* spp. or *L. monocytogenes* were evaluated for their applicability as binders for bead array and sandwich ELISA for detection of *L. monocytogenes* in buffer and in 11 different food types. The bead array format consistently demonstrated lower detection limits and was less affected by interference from food matrices than the sandwich ELISA format. However, the obtained detection limits were not sufficient to satisfy the required standard for *L. monocytogenes* testing. Therefore, the international organization for standardization (ISO 11290-1:1996) methods for pre-enrichment and enrichment were employed to increase the bacteria numbers. When compared to the standard plating method, the bead array was able to detect the bacteria with the same accuracy even at the 1 CFU level after only 24 h of the enrichment period. In addition, *Listeria*-specific 3C3 and *L. monocytogenes*-specific 7G4 antibodies were successfully employed to construct a multiplex detection for *Listeria*, *Salmonella* and *Campylobacter* in a bead array format by combining with commercial *Salmonella*-specific and available *Campylobacter*-specific antibodies.

1. Introduction

Among the six species in the genus *Listeria*, which include *Listeria monocytogenes*, *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri*, *Listeria ivanovii*, and *Listeria grayi* (Hitchins & Jinneman, 2011), only *L. monocytogenes* is pathogenic to humans, causing listeriosis which is characterized by sepsis, meningitis and encephalitis (Schuchat, Swaminathan, & Broome, 1991). The disease occurs primarily in infants, pregnant women, elderly patients, and immune compromised individuals (O'Neil & Marquis, 2006). However, healthy individuals can also suffer from gastroenteritis when they consume large amounts of the bacterium (Dalton et al., 1997). Although contamination with this bacterium is found less frequently than that for other common foodborne bacteria, the fatality rate from listeriosis has been estimated to be as high as 36% (Siegman-Igra et al., 2002). This bacterium also poses a serious threat to food safety because of its tolerance to stress conditions during food processing (Roberts & Wiedmann, 2003), its ability to grow at refrigerator temperature (Bayles, Annous, & Wilkinson, 1996), and its resistance to antibiotics (Prazak, Murano, Mercado, & Acuff, 2002).

Several culture and biochemical methods have been routinely used to distinguish human pathogenic *L. monocytogenes* from the other *Listeria* spp. (Brackett & Beuchat, 1989; McClain & Lee, 1988). However, variation in hemolytic activity among *L. monocytogenes* strains can compromise the accuracy of the test result (Conner, Scott, Sumner, & Bernard, 1989). Moreover, these tedious and time

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consuming identification methods are not suitable for screening large numbers of food samples required to meet increasing global demand. Consequently, high-throughput methods based on detecting nucleotide sequences that are unique to bacterial targets were developed, such as a combination of multiplex PCR and microarray-based detection (Volokhov, Rasooly, Chumakov, & Chizhikov, 2002). However, these nucleotide-based methods generally need sophisticated detectors, elaborate sample extraction, and highly-trained personnel. Therefore, immunoassays are still preferred by many end-users for high volume sample screening.

Immunoassay is an alternative method to screen for analytes in a large volume. However, the specificity and sensitivity of any immunoassay rely heavily on the characteristics of the antibody employed to perform molecular binding in the assay. Hence, there have been numerous efforts to produce specific antibodies that can distinguish *L. monocytogenes* from other *Listeria* spp. by using several immunogens such as listeriolysin O (Edelson, Cossart, & Unanue, 1999; Erdenlig, Ainsworth, & Austin, 1999; Nato et al., 1991), protein from cell membrane (Lathrop, Jaradat, Haley, & Bhunia, 2003), flagella (Kim et al., 2005), heat-killed *L. monocytogenes* (Heo, Nannapaneni, Story, & Johnson, 2007), and formalin-killed *L. monocytogenes* (Loiseau et al., 1995; Solve, Boel, & Norrung, 2000). However, the antibodies obtained from these libraries have not been able to distinguish *L. monocytogenes* from other *Listeria* spp.

Additional reports claim to have successfully produced antibodies specific to *L. monocytogenes*, or to its proteins, by using various antigens for the immunization. For instance, a study used formalin-killed *L. monocytogenes* as an immunogen to produce a *L. monocytogenes*-specific antibody, but not all *Listeria* spp. were tested in the cross reactivity test (Hearty, Leonard, Quinn, & O'Kennedy, 2006). Another group used a *L. monocytogenes* secreted p60 protein encoded by the *iap* (invasion-associated protein) gene as an immunogen in a hybridoma library production and found an antibody that was specific to the p60 protein of *L. monocytogenes*, but actual *L. monocytogenes* cells were not tested (Yu et al., 2004). Bhunia and Johnson (1992) used heat-killed *L. monocytogenes* as an immunogen to produce a *L. monocytogenes*-specific antibody which was characterized by antigen-trapped enzyme-linked immunosorbent assay (ELISA), dot blotting and colony blotting (Bhunia & Johnson, 1992). These previous reports have paved the way to demonstrate the possibility of producing a *L. monocytogenes*–specific antibody. However, most of the above antibodies were produced with applications involving specific immunoassay formats in mind, and none aimed to employ the selected antibodies in a multiplex detection format. Moreover, none of these studies have demonstrated the ability of the antibodies to detect the presence of the bacterium in food samples.

In this study, formalin-killed and heat-killed *L. monocytogenes* were used as immunogens to produce two hybridoma libraries in order to increase the possibility of identifying monoclonal antibodies highly specific to *L. monocytogenes*. These were subsequently characterized for their performance in a range of different immunoassay formats, including a bead array and sandwich ELISA. The feasibility of using these immunoassays for food testing was evaluated in a wide range of foods including vegetables, meats, and dairy products. In addition, to lower the detection limit, pre-enrichment and enrichment protocols according to the international organization for standardization (ISO 11290-1:1996) were employed to increase the number of bacteria before being tested on the bead array. The results were then compared to the standard plating method. Furthermore, the selected *Listeria*-specific antibodies from this study were employed in combination with *Salmonella*-specific and *Campylobacter*-specific antibodies to construct a multiplex detection of three foodborne pathogens (*Listeria*, *Salmonella* and *Campylobacter*) in a bead array format.

2. Materials and methods

2.1. Bacteria employed

All bacteria used in this study are summarized in Table 1. For *Listeria* spp., single colonies were inoculated in Tryptic Soy Broth supplemented with 0.6% of yeast extract (TSBYE) and incubated at either at 25 °C or 37 °C as specified (200 RPM, 18 h). The bacteria were washed twice with phosphate buffered saline (PBS, pH 7.4, 138 mM NaCl and 2.7 mM KCl) by centrifugation at 3000 RPM for 15 min at 4 °C and resuspended in sterile PBS. For heat-killed preparation, the bacterial suspension was heated at 80 °C for 1 h. For formalin-killed preparation, the bacterial suspension was incubated in 0.3% formalin in PBS at 25 °C for 24 h before the formalin was removed by washing with PBS. For irradiation, the bacterial solution was subjected to a 10 kGy dose of gamma radiation using a Gammabeam 650 irradiator located at the Agri-Food and Biosciences Institute for Northern Ireland, Belfast (UK). The irradiated bacteria were used as representatives of live bacteria because we were prohibited from using live bacteria in the laboratory facility hosting the Luminex machine used for bead array. It should be further noted that because the screening of the hybridoma library was performed at National Center for Genetic Engineering and Biotechnology (Thailand), whereas other experiments

Table 1
Bacteria used in this study.

Bacteriophage/Bacteria	Source	Tested in
Listeria monocytogenes	ATCC 19115	Screening
L. monocytogenes	NCTC 4885	Bead Array, ELISA
L.innocua	NCTC11288	Screening, Bead Array, ELISA
L.ivanovii	ATCC 700402	Screening
L.ivanovii	NCTC11846[d]	Bead Array, ELISA
*L.murrayi/L. gravi*subsp. *murrayi*	KU 9	Screening
*L.murrayi/L. gravi*subsp. *murrayi*	NCTC 10812[d]	Bead Array, ELISA
*L.grayi/L. gravi*subsp. *grayi*	ATCC 19120[d]	Bead Array, ELISA
L.seeligeri	NCTC 11856[d]	Bead Array, ELISA
L.welshimeri	Environmental swab[c]	Bead Array, ELISA
Salmonella Enteritidis	DMST 7106	Screening
S. Enteritidis	NCTC6676	Bead Array, ELISA
S. Typhimurium	ATCC 13311	Screening
S. Typhimurium	Pig carcass swab[a]	Bead Array, ELISA
S. Choleraesuis	DMST 5880	Screening
S. Dublin	Pork[a]	Bead Array, ELISA
S. Infantis	Raw chicken[a]	Bead Array, ELISA
S. Senftenberg	Animal feed[b]	Bead Array, ELISA
S. Hadar	QA sample- LGC[b]	Bead Array, ELISA
S. Mbandaka	Hygiene swab[a]	Bead Array, ELISA
S. Virchow	NCTC5742	Bead Array, ELISA
Escherichia coli O157:H7	DMST 12743	Screening, Bead Array, ELISA
Klebsiella pneumoniae	ATCC 27736	Screening
Bacillus cereus	BIOTEC	Screening
Campylobacter jejuni	ATCC 29425	Bead Array, ELISA

NCTC: National Collection of Type Cultures, Colindale, London, UK.
ATCC: American Type Culture Collection (Manassas, VA).
DMST: Department of Medical Sciences, Thailand.
KU: Kasetsart University, Thailand.
BIOTEC: National Center for Genetic Engineering and Biotechnology, Thailand.
[a] Originally isolated and serotyped by Salmonella Reference Laboratory, Agri-Food and Biosciences Institute for Northern Ireland, Belfast, UK and kindly provided by Dr. Robert Madden.
[b] Laboratory of the Government Chemist, Middlesex, UK.
[c] Originally isolated from environmental swabs taken at food processing facilities and serotyped by Teagasc Food Research Centre, Moorepark, Republic of Ireland. Kindly provided by Dr. Kieran Jordan.
[d] Kindly provided by Mr. Mark Linton, Agri-Food and Biosciences Institute for Northern Ireland, Belfast, UK.