A new competitive fluorescence immunoassay for detection of Listeria monocytogenes

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Analytical Methods

Cite this: *Anal. Methods*, 2012, **4**, 4187

www.rsc.org/methods

PAPER

A new competitive fluorescence immunoassay for detection of *Listeria monocytogenes*

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Received 5th September 2012, Accepted 13th October 2012
DOI: 10.1039/c2ay25997d

A new competitive immunoassay has been designed using a specific monoclonal antibody labeled with a fluorophore. A specific and highly conserved peptide of 11 amino acids from the protein p60 of *L. monocytogenes* has been synthesised and used for the production of a monoclonal anti-p60. This antibody was used in the conception of a detection test able to detect 1 CFU of *L. monocytogenes* after only 18 hours of incubation with a minimum of manipulation. The test is based on a competition principle between the recombinant p60 protein and the p60 from *L. monocytogenes* present in the sample. A column containing a sepharose matrix was used to immobilize the recombinant p60 protein and the labeled monoclonal antibody, which is captured by the p60 from the sample when added to the column. An increase of the fluorescence signal of the eluate means a positive result. No cross-reactivity was observed with non-pathogenic *Listeria* species and each serotype of *L. monocytogenes* can be detected whereas some other immunological methods show cross-reactivity and false negative.

Introduction

Listeria monocytogenes is an important foodborne pathogen involved in several outbreaks around the world resulting in considerable economic and human losses. Its widespread presence in the environment and its persistence in food plants lead to a labored control of this microorganism. *L. monocytogenes* is a Gram-positive, rod-shaped facultative anaerobic bacterium. This microbe can grow in a wide range of temperatures (1 °C to 45 °C) between pH 4.6 and 9.5 and at a water activity as low as 0.92.[1] It finds favorable growth conditions on floors, drains and equipment within food industry premises, notably in the cold and wet atmosphere of refrigerated rooms where only psychrotrophic bacteria can survive.[2] In a review published in 2004, few studies are mentioned in which persistence of *L. monocytogenes* is demonstrated.[3] Refrigerated and ready-to-eat foods constitute thereby a potential risk for contamination.[4,5] To counter cross-contamination, the control of the environment by detecting the bacteria on the working surfaces and instruments could be an important tool. Several well-known outbreaks and food recalls due to *L. monocytogenes*[6–9] combined with the high case-fatality rate of 20–30% (ref. 10) have increased the need for more rapid, sensitive, and specific methods for detection of this bacterium not only in food, but also in the working environment.

L. monocytogenes is a widespread microorganism that can be readily isolated from a number of sources, such as soil, water, meat, and vegetables.[11,12] Thus, the bacterium can easily be introduced in the human food chain and it becomes difficult to avoid contamination. Many reliable and accurate culture methods and media have been already developed for the detection of *L. monocytogenes.*[13,14] However, these methods remain laborious, time-consuming and involve complicated procedures.[15–19] Some standard methods for the detection of *L. monocytogenes* can require up to 7 days to yield results, as they rely on the ability of microorganisms to multiply to visible colonies.[13] Existing DNA detection based methods are also expensive.

To overcome the difficulty of recovering *L. monocytogenes* in the presence of non-pathogenic *Listeria* species and increase the sensitivity of the detection, several PCR-based methods have been developed. These PCR methods[14,18–20] provide sensitive, specific, and reproducible detection of pathogenic bacteria. Despite showing valuable advantages, the fact remains that the limitations of applying PCR techniques cannot be disregarded. Among them, there is the presence of inhibitory substances,[21,22] complexity of matrices, sample preparation and DNA extraction procedures.[14,23] Moreover, from an industrial point of view, routine detection of microbes using PCR can be expensive as it requires specialized equipment and qualified workers to carry out the tests. On the other hand, methods based on antigen–antibody bindings constitute a good alternative for detection of foodborne pathogens. The field of immunology-based methods provides very powerful analytical tools for a wide range of targets

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including bacterial cells, spores, viruses and toxins.[24] Only for *L. monocytogenes*, few immunology-based methods already exist.[25–34] Various antibody types and formats are available for immune-detection. These include conventional and heavy chain antibodies, as well as polyclonal, monoclonal or recombinant antibodies. While polyclonal antibodies are limited both in terms of their specificity and abundance, monoclonal antibodies are often more useful for specific detection because they provide an indefinite supply of single antibodies directed against a single and unique epitope. The progress of hybridoma techniques and the emergence of the recombinant antibody phage display technology have led to more sensitive, specific, reproducible and reliable immunological detection with many commercial immunoassays adapted for a variety of microbes and their products.[35]

The protein p60 of *L. monocytogenes*, which is encoded by the *iap* (invasion-associated protein) gene, is considered an important virulence factor, although the exact role of the protein is not completely known.[36,37] Mutants of *L. monocytogenes*, which impair synthesis of p60, show a rough-colony morphology (R mutants) and are strikingly attenuated in virulence in mice. These mutants have also lost the capability of invading 3T6 mouse fibroblasts and form particularly long cell chains. Treatment of these mutants with partially purified p60 from wild-type *L. monocytogenes* restores their invasiveness and cell morphology.

The p60 protein could be a potential target for immunological detection because of its high abundance in the culture supernatant and high immunogenicity.[31,38] Nevertheless, it has been shown that an antiserum raised against the whole p60 is not appropriate for specific detection of *L. monocytogenes*, since cross-reactivity occurred with p60-related proteins in the culture supernatant of all *Listeria* species.[39] For this reason, the production of antibodies using p60 protein should be directed against an epitope, which is specific to *L. monocytogenes*. Many attempts have been made to produce *Listeria*-specific antibodies, but in most cases the potential was limited by the cross-reactivity with non-pathogen *Listeria* species or by the non-recognition of certain *L. monocytogenes* strains, which is not suitable for specific immunodetection.[40] In a previous work, a short hydrophilic peptide of eleven amino acids (QQQTAPKAPTE) namely PepD has been identified within the p60 protein to be a highly conserved region specific to all *L. monocytogenes* strains.[39] The polyclonal antibodies raised against this peptide showed a highly specific recognition for the p60 protein of all stains of *L. monocytogenes* tested and no cross-reactivity with non-pathogen *Listeria* spp. The results of this experiment allow us to consider this small peptide a good target for the development of an immune-detection test. However, due to the possible low titers of the polyclonal antibodies, a large amount of the precipitated protein was necessary to perform the analysis. Moreover, from a standpoint of a manufacturer producing a detection test using these antibodies, the repeated production of enough polyclonal antibodies may be demanding. Considering all of these limitations, the production of a monoclonal antibody using the same peptide of eleven amino acids specific to the p60 of *L. monocytogenes* was chosen. The aim of this study was to develop a competitive fluorescence immunoassay for the detection of *L. monocytogenes* using a p60 monoclonal antibody of *L. monocytogenes*.

Material and methods

Synthetic peptide, monoclonal antibody and recombinant P60 production

The peptide synthesis and the production of the anti-P60 monoclonal antibody were carried out by the company GenScript USA Inc., NJ, USA. First, the antigenic peptide PepD (QQQTAPKAPTE) derived from p60 of *L. monocytogenes* (accession number AEO02672 in the NCBI protein database) was synthesized by stepwise solid-phase peptide synthesis (SPPS) with an additional N-terminal cysteine residue for coupling.[38] Synthetic peptide was purified by reverse-phase HPLC and coupled *via* the SH group of the N-terminal cysteine residue to the keyhole limpet hemocyanin (KLH) carrier protein in order to stimulate the immune response. Five Balb/c mice were inoculated by intraperitoneal injection with 25–100 μg of the conjugated peptide emulsified in the TiterMax adjuvant for primary immunization and boosted 3 times with 12.5–50 μg of the conjugated peptide, also emulsified in the TiterMax adjuvant on days 14, 35 and 63. Cell fusion, subcloning of positive parental clones and expansion of positive subclones were screened using ELISA. Monoclonal antibodies from the hybridoma culture supernatant were purified using the protein A/G affinity column. ELISA and Western blot were performed to determine the purity, the concentration and the reactivity of the final antibody. Recombinant P60 protein has also been produced by Genscript. The pUC57 DNA plasmid was transformed into competent *E. coli BL21 (DE3)*. The recombinant P60 protein was purified using the nickel column *via* polyhistidine-tag. Final protein concentration was found to be 0.531 mg ml^{-1} as determined by the Bradford protein assay with BSA as a standard. A purity of about 80% was estimated using Coomassie blue-stained sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Bacterial strains and growth conditions

Several strains of *Listeria* were used in this experiment: *L. monocytogenes* 2812, *L. monocytogenes* 1043 and *L. monocytogenes* 2569 (serotypes 1/2a), *L. monocytogenes* 2558, *L. monocytogenes* 2739 and *L. monocytogenes* 2371 (serotypes 1/2b), *L. innocua* LSPQ 3285 (purchased from Laboratoire de Santé Publique du Québec, Ste-Anne-de-Bellevue, Québec, Canada). 1 ml of the fresh culture of all the strains were subcultured in 9 ml of Tryptic Soy Broth (TSB, Difco Laboratory, Detroit, MI) at 37 °C under stirring. Culture supernatants were collected after centrifugation at 5500 rpm for 10 minutes followed by filtration using a 0.25 μm syringe filter (Sarstedt, Montreal, Quebec) in order to obtain cell-free supernatants.

ELISA experiments

Protein samples were prepared as follows: recombinant p60 protein was diluted with a coating buffer (9 mM N_2CO_3, 0.02 M $NaHCO_3$, 1 mM NaN_3, pH 9.6) to obtain a concentration of 1.0 μg ml^{-1}. For bacterial samples, 50 μl of cell-free supernatants from overnight cultures was diluted with 50 μl of the coating buffer. The coating of the recombinant protein and supernatants was performed in microplates (LockWell, Maxisorp from Nunc)

overnight at 4 °C (100 µl per well). After coating, the plates were washed three times (10 min per washing) with a washing buffer (PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) containing 0.05% Tween 20) and blocked with 5% skimmed milk in PBS for 1 hour at 37 °C. The plates were sequentially incubated with the monoclonal IgG anti-p60 (18 µg ml^{-1}) and then with a horseradish peroxidase (HRP) labelled secondary antibody (Biolynx Inc., Brockville, Ontario), diluted as directed by the supplier. Both antibodies were diluted with a diluting buffer (washing buffer containing 1% skimmed milk). For colorimetric reaction, 100 µl of the enzyme substrate 3,3′,5,5′-tetramethylbenzidine (TMB) (Biolynx Inc., Brockville, Ontario) was added and the plates were incubated for 10 minutes at room temperature. Absorbance at 450 nm was measured using a microplate reader after the addition of 50 µl of 2 M H_2SO_4. For all ELISA experiments, two negative controls were performed using a sample of BSA and fresh TSB culture media (data not shown). A second ELISA was done to validate the competition principle of the test. An amount of 0.1 µg of recombinant p60 protein diluted in 100 µl of the coating buffer was primarily coated in each well overnight at 4 °C. After coating, the plates were washed three times (10 min per washing) with the washing buffer and blocked with 5% skimmed milk in PBS for 1 hour at 37 °C. Monoclonal IgG anti-p60 (18 µg ml^{-1}) was then added in each well and incubated for 1 hour at 37 °C. After another washing step, samples of 100 µl of each cell-free supernatant from the different *Listeria* strains were incubated in wells for 15 minutes at room temperature. The assay was then finalized as described above with the secondary antibody and the TMB substrate.

Western blot experiment

Cell-free supernatants from overnight cultures (4 ml) were concentrated by a factor of 66 after filtration through a 30 kDa Amicon Ultra centrifugal filter unit (Millipore, Ontario, Canada), diluted 1 : 1 with the Laemmli sample buffer (Bio-Rad Laboratories, Ontario, Canada) and heated at 100 °C for 5 minutes. Protein separation was achieved by SDS-PAGE in 10% polyacrylamide gels. Transfer onto nitrocellulose membranes was performed overnight at 4 °C. Membranes were blocked for 1 hour at room temperature under stirring in blocking buffer (PBS containing 5% skimmed milk). After three washings with PBS 0.05% Tween 20 (10 min per washing under stirring), the membranes were incubated with monoclonal IgG anti-p60 (0.4 µg ml^{-1}) for 1 hour at room temperature, washed three times as described above and then incubated with a horseradish peroxidase (HRP) labelled secondary antibody (Biolynx Inc.), diluted as directed by the supplier, for 1 hour at room temperature. The membranes were washed three times and then developed with the western blotting detection reagent Amersham ECL before being exposed to a photographic film (GE Healthcare Life Sciences, Québec, Canada). Recombinant P60 protein was used as a positive control.

Labeling of monoclonal IgG

A 0.5 ml sample of monoclonal anti-p60 IgG in 50 mM sodium borate buffer, pH 8.5 (2.0 mg ml^{-1}) was labelled using a Dylight 550 Antibody labeling kit (Thermo Fisher Scientific Inc., IL, USA). The labeling was achieved according to the supplier instructions. The degree of labelling (DOL), as calculated from the absorbance values at $\lambda = 280$ and 557 nm by applying a correction factor label absorption at $\lambda = 280$ nm, was found to be 2.88.

Affinity column preparation

The affinity column was obtained by conjugating the recombinant p60 protein with activated CH-Sepharose 4B (Sigma, Deisenhofen, Germany) as follows. One gram of dry resin powder was suspended in 200 ml of cold 1 mM HCl. The swollen resin was filtered in a polystyrene column (4 ml) (Bio-Rad Laboratories, Mississauga, Ont.) and washed with cold 1 mM HCl. A 3 ml sample of the p60 protein (0.531 mg ml^{-1}) was dialysed in 1 litre of 0.1 M sodium bicarbonate, pH 8.0, containing 0.5 M NaCl overnight at 4 °C using a dialysis membrane of 3500 Da. Dialysed protein was mixed with the resin at 4 °C overnight under slow inversion. After the coating, excess active groups were blocked with 0.1 M Tris–HCl buffer, pH 8.0 for 1 hour. To remove the unbound protein, the resin was washed successively with cold 0.1 M sodium bicarbonate (30 ml), 0.05 M Tris–HCl, 0.5 M NaCl, pH 8.0 (30 ml), 0.05 M sodium acetate, 0.5 M NaCl, pH 4.0 (30 ml), PBS, pH 7.4 (30 ml) and stored in PBS containing 0.05% sodium azide at 4 °C. The stored column was then washed 5 times with PBS. A 0.5 ml sample of labeled monoclonal IgG and 1.5 ml of PBS were added to the resin and incubated overnight at 4 °C under inversion to fix the IgG on the recombinant p60 protein. The resin was washed with PBS until the fluorescence signal returned to the minimum level ($\lambda = 550$ nm excitation, $\lambda = 576$ nm emission).

Detection of *L. monocytogenes*

1 ml of the fresh culture of each bacterial strain was sub-cultured in 10 ml of TSB for 18 hours at 37 °C and successively diluted by serial dilutions with sterile physiological water (0.85% NaCl). 0.1 ml of each dilution was plated on trypticase soy agar (TSA, Difco Laboratory, Detroit, MI) in order to achieve a bacterial count. The right volume was calculated to obtain a theoretical amount of 1 CFU that has been inoculated in 10 ml of TSB for another 18 hours at 37 °C. The cell-free supernatant from this last 18 hours culture (2 ml) was incubated with the resin for 10 minutes at room temperature under inversion. At the end of the incubation, 2 ml of the flow-through containing the free p60–IgG–fluorophore complexes was harvested from the column. The fluorescence emission signal of this flow-through was measured at $\lambda_{em} = 576$ nm with an excitation wavelength of $\lambda_{ex} = 550$ nm and compared with the fluorescence signal of the background which corresponds to the residual fluorescence signal after the last wash of the column before the incubation with the bacterial sample. The column was then washed several times with PBS until the fluorescence signal returned to a minimum and constant level before being reused for another assay.

Results and discussion

Western blot analysing with monoclonal IgG anti-p60

The anti-p60 produced was tested to determine whether it was suitable for the specific detection of the *L. monocytogenes* p60

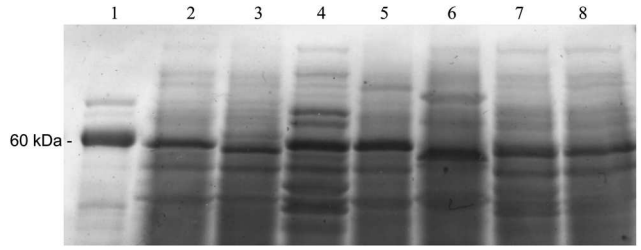

Fig. 1 Protein separation of concentrated cell-free supernatants from overnight cultures of various *Listeria* species by 10% SDS-PAGE electrophoresis. Lanes: 1, recombinant p60 protein; 2, *L. monocytogenes* HPB 2569; 3, *L. monocytogenes* HPB 2558; 4, *L. monocytogenes* HPB 1043; 5, *L. monocytogenes* HPB 2812; 6, *L. monocytogenes* HPB 2739; 7, *L. monocytogenes* HPB 2371; 8, *L. innocua*.

protein. For this purpose, a Western blot analysis with the supernatant proteins of various *Listeria* strains was performed. The six strains of *L. monocytogenes* used for the experiment belong to two of the three serotypes responsible for up to 96% of the cases of human listeriosis.[41] *L. innocua* was used as non-pathogenic *Listeria* species. The protein pattern of the culture supernatants obtained by SDS-PAGE is shown in Fig. 1. A 60 kDa protein band is clearly visible for each of the strains which correspond to the protein p60 or the p60-related protein of *L. innocua*.[38] The important background on the gel and the large amount of protein bands are due to the fact that supernatants concentrated by a factor of 66 were used to reach a sufficient concentration of the p60 protein that allowing its visualization on the gel. As shown in Fig. 2, the monoclonal anti-p60 reacted specifically with the p60 protein of *L. monocytogenes* whereas no cross reactivity with the p60-related protein of *L. innocua* could be observed. These results are consistent with those obtained by Bubert *et al.* (1992). It can be assumed, based on the results, that the monoclonal antibody used in this study, raised against the same PepD, is highly specific and can recognise all of the 13 serotypes of *L. monocytogenes* evaluated in this study.

Detection of the native p60 protein in supernatants by ELISA

While the Western blot analysis allows verifying the recognition of denatured proteins by antibodies, an ELISA test performed with fresh supernatants allows verifying the recognition of native proteins. The possibility that the epitope is inaccessible when the

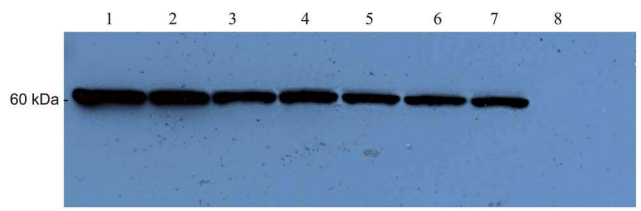

Fig. 2 Western blot analysis of concentrated cell-free supernatants from overnight cultures of various *Listeria* species using a monoclonal anti-p60 antibody. Lanes: 1, recombinant p60 protein; 2, *L. monocytogenes* HPB 2569; 3, *L. monocytogenes* HPB 2558; 4, *L. monocytogenes* HPB 1043; 5, *L. monocytogenes* HPB 2812; 6, *L. monocytogenes* HPB 2739; 7, *L. monocytogenes* HPB 2371; 8, *L. innocua*.

Table 1 Reactivity of the monoclonal anti-p60 with supernatants from different *Listeria* strains by direct ELISA

Organism	Optical density
Listeria innocua	0.088
Listeria monocytogenes	
HPB 2812	1.022
HPB 1043	0.961
HPB 2569	1.123
HPB 2558	0.962
HPB 2739	1.113
HPB 2371	0.865

protein is in its native form cannot be excluded. Therefore, it is important to perform both of these tests in order to confirm the detection of the epitope in the native and denatured form. Direct ELISA tests were performed with fresh cell-free culture supernatants of the different strains of *Listeria*. Since ELISA is more sensitive than the Western blot, it was achieved with non-concentrated supernatants. Results are shown in Table 1 and it can be seen that the monoclonal anti-p60 recognized all the *L. monocytogenes* strains tested while it presented no cross-reaction with the supernatant of non-pathogenic *L. innocua*. The recombinant p60 protein was used as a positive control, and fresh TSB broth alone, used as a negative control, did not show any reaction (data not shown).

Validation of the principle of the test by ELISA

The detection test is based on a principle of competition between the recombinant p60 protein, to which the labeled monoclonal anti-p60 is fixed in the column, and the p60 protein secreted by bacterial strains. The determinant factor that underpins the success of the test is the ability of these fixed antibodies of being naturally displaced by competition on the free p60 protein from the bacterial culture sample after its injection into the column. The amount of antibodies transferred depends on the concentration of the free p60 protein. The new antibody-free p60 protein complexes can be harvested at the end of the column and the fluorescence rate is measured. In order to verify whether the antibodies can be transferred, a competition ELISA has been designed to recreate what happens in the column. However, it is not a classical competition ELISA since both of the antigens were not added at the same time. First, 0.1 μg of the recombinant p60 protein was coated in each well on which a certain amount of antibodies was then fixed. A volume of the supernatant from the *Listeria* culture was added to each well and incubated for 15 minutes at room temperature to allow the antibodies to get transferred on the free p60 protein from supernatants. These new antibody-free p60 protein complexes were washed and the lost in the initial amount of antibodies is revealed by a diminution of the optical density after the reaction with a secondary antibody. The results of this competition ELISA show that supernatants obtained from *L. monocytogenes* cultures can dislodge antibodies from their initial position while the supernatant from *L. innocua* cannot (Table 2). It means that the antibodies recognise only the p60 protein in the supernatant of the *L. monocytogenes* strains and that this protein can capture a certain amount of antibodies already fixed on the recombinant p60 protein. The same

Table 2 Non-classical competition ELISA[a] designed to visualise the displacement of the monoclonal anti-p60 from the immobilized recombinant p60 protein to the free p60 protein from supernatants

Supernatant added	Optical density
None	1.239
Recombinant p60 (0.1 μg)	0.623
L. innocua	1.241
L. innocua diluted	1.229
L. monocytogenes	
HPB 2812	0.661
HPB 2812 diluted	0.996
HPB 1043	0.593
HPB 1043 diluted	0.983
HPB 2569	0.701
HPB 2569 diluted	1.086
HPB 2558	0.638
HPB 2558 diluted	1.018
HPB 2739	0.777
HPB 2739 diluted	1.070
HPB 2371	0.663
HPB 2371 diluted	1.069

[a] First, 0.1 μg of the recombinant p60 protein was coated in each well on which a certain amount of antibodies was then fixed. A volume of supernatant from the *Listeria* culture was added to each well and incubated for 15 minutes at room temperature to allow the antibodies to get transferred on the free p60 protein from supernatants. The lost in the initial amount of antibodies is revealed by a diminution of the optical density after the reaction with the secondary antibody.

experiment was also performed with supernatants diluted to half to see if the diminution of the optical density would be less important. This is indeed what has been observed and all these results confirm that the competition principle can be applied in the column for the detection test.

Detection of *L. monocytogenes*

The preparation of the column was carried out by fixing the labeled monoclonal anti-p60 to the recombinant p60 protein, which is immobilized on a Sepharose 4B matrix. The detection of the bacteria is possible due to the displacement of the labeled monoclonal anti-p60 by the p60 protein secreted by *L. monocytogenes*. Displaced fluorescent antibodies are measured by fluorescence emission of the eluate and represent a measure of the amount of free p60 protein in the supernatant and thus, if the initial sample was contaminated by *L. monocytogenes*. Before testing any sample, the column was washed several times with a neutral buffer until the fluorescence signal of the eluate was minimal and constant. This signal was considered as the background. A volume (2 ml) of cell-free supernatants from an 18 hour culture inoculated with a theoretical amount of 1 CFU was added to the column and incubated for 10 minutes at room temperature. A 2 ml eluate fraction was collected and the fluorescence emission was measured and compared with the background signal. A significant increase in the fluorescence signal means that *L. monocytogenes* was present in the initial sample. The increase of fluorescence signal for each bacterial strain is shown in Fig. 3. For each strain of *L. monocytogenes* tested, a significant increase in fluorescence emission was observed compared to the background whereas the signal for *L. innocua* remained constant.

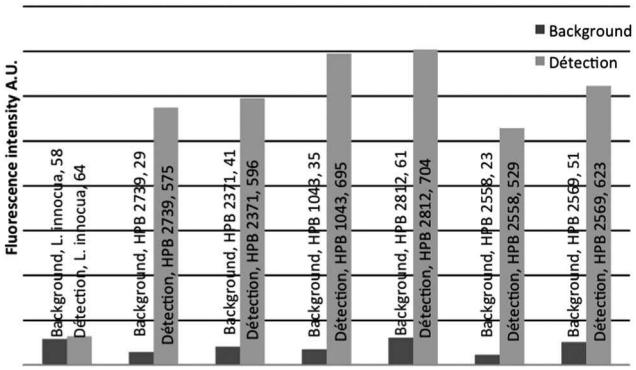

Fig. 3 Fluorescence immunoassay for detection of *L. monocytogenes*. Fluorescence emission was recorded at $\lambda_{em} = 576$ nm and is a representative of the labeled antibody eluted from the column after incubation with cell-free supernatants from overnight cultures of various *Listeria* species. Background signals represent the minimal and constant fluorescence emission after several washes. Excitation wavelength was at $\lambda_{ex} = 550$ nm.

Conclusions

In conclusion, a monoclonal antibody has been produced using a small 11 amino acid peptide of which the sequence is highly conserved in the protein p60 of *L. monocytogenes*. Our results showed that this antibody is very specific to the p60 protein of *L. monocytogenes* and presents no cross-reactivity with the p60-related protein of non-pathogenic *Listeria* species. This antibody was successfully used in the conception of a competitive fluorescent immunoassay for the detection of *L. monocytogenes*. The method consisted of the fixation of the labeled monoclonal anti-p60 on a recombinant p60 protein, which is immobilized on a Sepharose 4B matrix. The addition of a cell-free supernatant from a bacterial culture containing the secreted p60 protein induces the displacement of the antibody, which results in an increase in the fluorescence signal of the eluate at the emission wavelength of the fluorophore. The assay allowed the detection of the 6 strains of *L. monocytogenes* tested after only 18 hours of incubation with a theoretical initial inoculum of 1 CFU. No false positive with *L. innocua* was observed and thus, this assay represents a promising way to develop a sensitive, specific and rapid detection test not only for *L. monocytogenes*, but also for many other pathogens. Other experiments are in progress for the optimisation of the test and also for the establishment of a standardized procedure applicable in the industry.

Acknowledgements

This research was supported by the Ministry of Economic Development, Innovation and Export Trade, International Program (MDEIE). S. Beauchamp was also supported by a fellowship from Nature Research and Technology: International Training Program. The project was also partially funded by the CNR project "Conoscenze integrate per sostenibilità e innovazione del Made in Italy agroalimentare (CISIA)" to A.P. and S.D.

References

1 M. Gandhi and M. L. Chikindas, *Int. J. Food Microbiol.*, 2007, **113**, 1–15.

2 B. Carpentier and O. Cerf, *Int. J. Food Microbiol.*, 2011, **145**, 1–8.
3 T. Møretrø and S. Langsrud, *Biofilms*, 2004, **1**, 107–121.
4 S. D. Ha, S. Y. Park, J. W. Choi, J. Yeon and M. J. Lee, *et al.*, *J. Microbiol. Biotechnol.*, 2005, **15**, 1323–1329.
5 C. W. Donnelly, *Nutr. Rev.*, 2009, **59**, 183–194.
6 P. D. MacDonald, R. E. Whitwam, J. D. Boggs, J. W. Reardon and R. R. Saah, *et al.*, *Clin. Infect. Dis.*, 2001, **33**, 1236.
7 S. J. Olsen, M. C. Evans, S. Hunter, V. Reddy and L. Kornstein, *et al.*, *Clin. Infect. Dis.*, 2001, **33**, 1237.
8 K. C. Klontz, S. Wong, D. Street and S. I. Delgado, *J. Food Prot.*, 2000, **63**, 1113–1116.
9 E. C. D. Todd and S. Notermans, *Food Control*, 2011, **22**, 1484–1490.
10 B. Swaminathan and P. Gerner-Smidt, *Microbes Infect.*, 2007, **9**, 1236–1243.
11 M. Magnani, G. Amagliani, G. Brandi, E. Omiccioli and A. Casiere, *et al.*, *Food Microbiol.*, 2004, **21**, 597–603.
12 D. H. Chung, W. B. Shim, J. G. Choi, J. Y. Kim and Z. Y. Yang, *et al.*, *J. Microbiol. Biotechnol.*, 2007, **17**, 1152–1161.
13 V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa and C. Adley, *Biotechnol. Adv.*, 2010, **28**, 232–254.
14 M. Zunabovic, K. J. Domig and W. Kneifel, *LWT–Food Sci. Technol.*, 2011, **44**, 351–362.
15 D. Y. Hao, L. R. Beuchat and R. E. Brackett, *Appl. Environ. Microbiol.*, 1987, **53**, 955–957.
16 D. McClain and W. H. Lee, *J. - Assoc. Off. Anal. Chem.*, 1988, **71**, 660–664.
17 S. B. Barbuddhe, V. S. Parihar, M. L. Danielsson-Thm and W. Tham, *Food Control*, 2008, **19**, 566–569.
18 M. Drake, J. Isonhood and L. A. Jaykus, *Food Microbiol.*, 2006, **23**, 584–590.
19 K. Balakrishna, H. S. Murali and H. V. Batra, *J. Food Saf.*, 2010, **30**, 263–275.
20 Z. G. Li, W. Chen, Y. W. Yang and W. Deng, *J. Food, Agric. Environ.*, 2010, **8**, 96–99.
21 M. C. Simon, D. I. Gray and N. Cook, *Appl. Environ. Microbiol.*, 1996, **62**, 822–824.
22 P. G. Lantz, B. Hahn-Hägerdal and P. Rådström, *Trends Food Sci. Technol.*, 1994, **5**, 384–389.
23 A. Agersborg, R. Dahl and I. Martinez, *Int. J. Food Microbiol.*, 1997, **35**, 275–280.
24 S. S. Iqbal, M. W. Mayo, J. G. Bruno, B. V. Bronk and C. A. Batt, *et al.*, *Biosens. Bioelectron.*, 2000, **15**, 549–578.
25 C. S. Chen and R. A. Durst, *Talanta*, 2006, **69**, 232–238.
26 R. L. Churchill, H. Lee and J. C. Hall, *J. Microbiol. Methods*, 2006, **64**, 141–170.
27 V. Gangar, M. S. Curiale, A. D'Onorio, A. Schultz and R. L. Johnson, *et al.*, *J. AOAC Int.*, 2000, **83**, 903–918.
28 K. Hibi, A. Abe, E. Ohashi, K. Mitsubayashi and H. Ushio, *et al.*, *Anal. Chim. Acta*, 2006, **573–574**, 158–163.
29 J. A. Hudson, R. J. Lake, M. G. Savill, P. Scholes and R. E. McCormick, *J. Appl. Microbiol.*, 2001, **90**, 614–621.
30 Y. S. Jung, J. F. Frank and R. E. Brackett, *J. Food Prot.*, 2003, **66**, 1283–1287.
31 K. Y. Yu, Y. Noh, M. Chung, H. J. Park and N. Lee, *et al.*, *Clin. Diagn. Lab. Immunol.*, 2004, **11**, 446–451.
32 M. Magliulo, P. Simoni, M. Guardigli, E. Michelini and M. Luciani, *et al.*, *J. Agric. Food Chem.*, 2007, **55**, 4933–4939.
33 A. M. Sewell, D. W. Warburton, A. Boville, E. F. Daley and K. Mullen, *Int. J. Food Microbiol.*, 2003, **81**, 123–129.
34 W. B. Shim, J. G. Choi, J. Y. Kim, Z. Y. Yang and K. H. Lee, *et al.*, *J. Microbiol. Biotechnol.*, 2007, **17**, 1152–1161.
35 P. Leonard, S. Hearty, J. Brennan, L. Dunne and J. Quinn, *et al.*, *Enzyme Microb. Technol.*, 2003, **32**, 3–13.
36 A. Bubert, M. Kuhn, W. Goebel and S. Kohler, *J. Bacteriol.*, 1992, **174**, 8166–8171.
37 M. Kuhn and W. Goebel, *Infect. Immun.*, 1989, **57**, 55–61.
38 A. Bubert, P. Schubert, S. Kohler, R. Frank and W. Goebel, *Appl. Environ. Microbiol.*, 1994, **60**, 3120–3127.
39 A. Bubert, S. Kohler and W. Goebel, *Appl. Environ. Microbiol.*, 1992, **58**, 2625–2632.
40 S. Hearty, P. Leonard, J. Quinn and R. O'Kennedy, *J. Microbiol. Methods*, 2006, **66**, 294–312.
41 R. B. Tompkin, *J. Food Prot.*, 2002, **65**, 709–725.

