RESEARCH PAPER

Rapid colorimetric detection of *Salmonella typhimurium* using a selective filtration technique combined with antibody–magnetic nanoparticle nanocomposites

**Won-Bo Shim · Jeong-Eon Song · Hyoyoung Mun ·
Duck-Hwa Chung · Min-Gon Kim**

Abstract Detection of pathogenic bacteria that pose a great risk to human health requires a rapid, convenient, reliable, and sensitive detection method. In this study, we developed a selective filtration method using monoclonal antibody (MAb)–magnetic nanoparticle (MNP) nanocomposites for the rapid and sensitive colorimetric detection of *Salmonella typhimurium*. The method contains two key steps: the immunomagnetic separation of the bacteria using MAb–MNP nanocomposites and the filtration of the nanocomposite-bound bacteria. Color signals from the nanocomposites remaining on the membrane were measured, which reflected the amount of bacteria in test samples. Immunomagnetic capture efficiencies of 8 to 90 % for various concentrations of the pathogen (2×10^4–2×10^1 cells) were obtained. After optimization of the method, 2×10^1 cells of *S. typhimurium* in pure culture solution was detectable as well as in artificially inoculated vegetables (100 cells/g). The method was confirmed to be highly specific to *S. typhimurium* without cross-reaction to other pathogenic bacteria and could be concluded within 45 min, yielding results in a shorter or similar time period as compared with recently reported antibody immobilized on magnetic-particle-based methods. This study also demonstrated direct application of MAb–MNP nanocomposites without a dissociation step of bacteria from magnetic beads in colorimetric assays in practice.

Keywords Antibody–magnetic nanoparticle · Colorimetric detection · Selective filtration · *Salmonella typhimurium*

Introduction

Food-borne pathogenic bacteria have posed a serious risk to human and public health and have been the cause of food poisoning in many countries. Therefore, earlier and highly sensitive detection of pathogenic microorganisms is critical, because even the lowest concentration of bacteria present in food rapidly propagate before consumption of the food [1]. In 2011, the World Health Organization published that 76 million cases of food-borne disease resulted in 325,000 patients and 5,000 deaths [2]. *Salmonella typhimurium* is one of the most frequently occurring pathogens associated with foods and food poisoning [3] and is responsible for 1.4 million cases of human illness, which is a significant portion (30 %) of all reported food poisonings in the United States [4, 5]. During recent decades, identification of *S. typhimurium* has been achieved through traditional culturing methods and immunological and genetic methods. Traditional cultivation methods are still considered as standard methods for the analysis of food-borne pathogenic bacteria. However, these conventional methods are time-consuming and laborious, with culturing requiring up to 5 days to yield results, and are cumbersome, and analysis of results on a large scale can be challenging. In addition, initial enrichment and other complicated procedures are also required [6].

Even though immunological methods provide rapid, specific, reproducible, and reliable detection of target bacteria, these methods still suffer from complicated steps in sample preparation and measuring procedures, such as pre-cultivation, incubation, and washing [7]. On the other hand, polymerase chain

Won-Bo Shim and Jeong-Eon Song contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-013-7497-6) contains supplementary material, which is available to authorized users.

W.-B. Shim · J.-E. Song · H. Mun · M.-G. Kim (✉)
School of Physics and Chemistry, Gwangju Institute of Science and Technology, Gwangju 500-712, Republic of Korea
e-mail: mkim@gist.ac.kr

D.-H. Chung
Division of Applied Life Science (BK 21 program), Graduate School, Gyeongsang National University, Jinju, Gyeongnam 660-701, Republic of Korea

reaction (PCR) has been regarded as a powerful technique to detect food pathogens, but it also requires purification of microorganisms by pre-cultivation, a number of experimental steps, such as DNA extraction, amplification, and electrophoresis, as well as well-trained analysts [8]. These methods described above commonly require pre-cultivation steps for the detection of *S. typhimurium* in food. Therefore, rapid sample preparation and isolation of pathogens from food are key points in the development of rapid methods for the detection of food-borne pathogens. Efficient and rapid isolation methods based on magnetic nanoparticles (MNP) or microbeads conjugated with antibodies specific to pathogens have been suggested. The methods are generally termed immunomagnetic separation, and they have been widely used to rapidly isolate and concentrate food pathogens for the diagnostic methods, such as PCR and immunoassays [7, 9–13].

In recent years, immunomagnetic separation combined with fluorescent and gold nanoparticles has been used directly for the rapid and facile detection of pathogenic bacteria [1, 14, 15]. In our previous study [16], we reported a selective filtration method using magnetic nanocomposites (antibody/gold nanoparticle/MNPs) for the detection of *Staphylococcus aureus* in milk (limit of detection: 150 CFU). In the selective method, there are two key steps, viz., the immunomagnetic separation of *S. aureus*, and the concentration of the *S. aureus*-bound nanocomposites on a nitrocellulose membrane. The immunomagnetically collected solution is poured onto a nitrocellulose filter through a polydimethylsiloxane (PDMS) film possessing six holes with 3 mm diameter and then vacuum-filtered. Through this process, the pathogen-bound nanocomposites are concentrated on the membrane and can then be colorimetrically measured, while unbound nanocomposites are passed through the membrane.

In the present study, this selective filtration method was expanded into the detection of *S. typhimurium*. Although the assay strategy is similar to that described in our previous report for the detection of *S. aureus* [16], in this study, we designed a simple nanocomposite with MNP and MAb which is newly prepared in our lab and specific to *S. typhimurium*. This new approach did not require a dissociation step to separate the target bacteria from the immuno-magnetic bead which can affect the interaction between antibodies and target in other analytical methods and then allowed the detection of *S. typhimurium* at a level of less than spiked vegetables with 10^2 cells/g within 45 min.

Materials and methods

Reagents and materials

MNPs with mean diameters of 50 and 100 nm were purchased from Chemicell Inc. (Berlin, Germany) and bovine serum albumin (BSA) was purchased from Fitzgerald Inc. (North Acton, MA, USA). Tween-20, sodium chloride, sodium tetraborate, and boric acid were obtained from Sigma (St. Louis, MO, USA). A magnet for magnetic nanoparticle separation was purchased from Invitrogen (Grand Island, NY, USA).

A MAb was produced from 4H3-6 hybridoma cells that were developed by cell fusion using myeloma cells and spleen cells obtained from mice that had been immunized with formalin-killed *S. typhimurium* in our laboratory. A protein G column used for the purification of MAb and 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDC) were obtained from Thermo Fisher Scientific Inc. (Rockford, IL, USA).

Nitrocellulose membranes with a 1.2 μm pore size was purchased from Whatman (Kent, UK), mixed cellulose ester membrane with a 1.2 m pore size, and acetate cellulose membrane with a 0.8 μm pore size were obtained from Advantec (Tokyo, Japan), and Asymmetric Super Micron Polysulfone (MMM) filter membrane with a 0.8 μm pore size was purchased from PALL (East Hills, NY, USA). A PDMS film with 3 mm diameter holes (six on each film), which can accumulate MAb–MNP bound to *S. typhimurium* onto filter membranes, was prepared in our laboratory by mixing Sylgard 184 A and B obtained from Sewang Hitech (Kimpo, Korea).

Phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.2) and 1 M Tris–HCl (pH 7.4) were purchased from Bioseasang (Seongnam, Korea). The deionized water used in all experiments was purified with a Purelab Option Water Purification System (ELGA, Marlow, UK).

Microorganism and culture conditions

S. typhimurium and other food-borne bacteria such as *Listeria monocytogenes* (ATCC 19112), *S. aureus* (ATCC 23235), *Escherichia coli* (ATCC 25922), and *Bacillus cereus* (KCTC 1092) were used in this study. These bacteria were grown in tryptic soy broth (Difco Laboratories, Franklin Lakes, NJ, USA) at 37 °C with gentle shaking at 150 rpm for 18 h. The cultured bacteria (1 mL) were collected by centrifugation at 6,000 rpm for 5 min. The supernatant was discarded, and the pellet was dispersed in 1 mL of autoclaved PBS. The centrifugation and washing steps were repeated twice, and the supernatant was discarded. Finally, the cell count was adjusted to 4×10^8 cells/mL in the autoclaved PBS, serially diluted, and used for a selective filtration.

Preparation of MAb–MNP nanocomposites

Two kinds (50 and 100 nm) of carboxylated MNPs were used to conjugate to the MAb specific to *S. typhimurium*. Briefly,

both MNPs (0.4 mL) was mixed with 0.15 mL of EDC (0.4 M) and incubated for 5 min at room temperature with rotation at 38 rpm on a rotary shaker. The MAb (20 μg) was added to the mixture and incubated for 2 h at room temperature with the same rotation. The surface residues on MNPs were blocked with 500 μL of 1 % BSA for 1 h at room temperature. The MAb–MNP nanocomposites were isolated using a magnet and washed with PBS. After two washes, the conjugate was dispersed in storage buffer (1 % BSA in PBS), filtrated through a 0.45 μm syringe filter (Advantec, Tokyo, Japan) to remove aggregated MNPs, and stored at 4 °C until required for use.

The capture efficiency of MAb–MNP for *S. typhimurium* (2×10^1, 2×10^2, 2×10^3, and 2×10^4 cells) was determined. After the isolation of cell–MAb–MNP complexes on the magnet, the complexes were spread onto xylose lysine deoxycholate agar (XLD) agar (Difco) plates. The colonies on the agar plates were counted after incubation for 24 h at 37 °C.

Development of the selective filtration system for *S. typhimurium*

A 0.2-mL aliquot of fresh *S. typhimurium* culture was transferred into a 1.5 mL Eppendorf tube, and the cells were prepared as described above. Ten microliters of the MAb–MNP diluted 1/20 in PBS was subsequently added to the suspended solution and incubated for 30 min at room temperature with gentle agitation. After incubation, the tubes were placed in a magnet (Invitrogen) for 10 min. During this time, the magnet was inverted several times to concentrate nanocomposites (bacteria–MAb–MNP) into a pellet on the side of the tube. The supernatant was removed, and cell–MAb–MNP complexes were washed with PBS and recovered with the magnet as described above. Final pellet was re-suspended in 0.2 mL of the PBS.

A filter membrane with a 0.8 to 1.2 μm pore size was wet with 0.05 % PBS containing 0.05 % Tween 20 (PBST) before being placed on a 1-L Erlenmeyer flask equipped with a filter. A PDMS film was placed onto the membrane to define the locations of the six holes on the PDMS film. The suspended cell–MAb–MNP (200 μL) was transferred into the holes through tubes under vacuum, and the solution was filtered through the filter membrane (Fig. 1). When cells bind to the MAb–MNP nanocomposites, the size of the complex (bacterial cell–MAb–MNP) increases such that it cannot be passed through the pores of the nitrocellulose filter. However, MAb–MNP nanocomposites unbound to bacteria can easily pass through the filter membrane. The color intensity on the membrane was quantified and averaged for each sample using a ChemiDocTM MP System (Bio-Rad, Hercules, California, USA), which converted the color signal to optical density values.

Specificity of the selective filtration system

To determine the specificity of the approach, the selective filtration was carried out with *S. typhimurium* and other bacteria, such as *L. monocytogenes*, *S. aureus*, *E. coli*, and *B. cereus* (all adjusted to 2×10^4 cells). The bacteria were cultivated and washed with PBS as described above and used to investigate the specificity of the selective filtration method.

### Analysis of vegetable samples artificially inoculated with *S. typhimurium*

Lettuce and cabbage were washed with deionized water, soaked in 20 % ethanol to sterilize, and cut into small sections of equal size. Samples (1 g) were placed into 15 mL conical tubes and artificially inoculated with *S. typhimurium* (0, 10^2, 10^4, 10^6, and 10^8 cells/g). The inoculated samples were maintained at room temperature for 2 h to absorb the bacteria onto the surface of the lettuce and cabbage samples. Five milliliters of autoclaved PBS was added to the samples, which were then shaken vigorously for 5 min; 1 mL of the extract was centrifuged at 6,000 rpm for 5 min, and the supernatant was discarded. Pellets were re-suspended in 1 mL of autoclaved PBS, and 200 μL of suspended samples was transferred into new e-tubes and mixed with 10 μL of MAb–MNP nanocomposites. The samples were incubated and treated as described above and subjected to the selective filtration system.

Results and discussion

Confirmation of MAb–MNP nanocomposites

Nano- and micro-sized magnetic particles are generally used for immunomagnetic separation of bacteria. In this study, we chose magnetic nanoparticles with 50 and 100 nm diameters, so that these nanoparticles could readily pass through membranes with 0.8 to 1.2 μm pore sizes, while the membranes could capture bacteria. In preliminary tests for the conjugation of MNP and MAb, MNP with a diameter of 50 nm often aggregated by themselves during synthesis, but conjugation with MNP (100 nm diameter) was readily performed. MNP coupled with BSA (BSA–MNPs) was used as a control for the MAb–MNP nanocomposites. The sizes of bare MNPs, BSA–MNPs, and MAb–MNPs were determined by dynamic light scattering (Otsuka Electronic Co., Osaka, Japan). As shown in Fig. S1 (Electronic supplementary material), the sizes of the particles gradually increased in the order of bare MNPs (diameter, 142 nm), BSA–MNPs (diameter, 211 nm), and MAb–MNPs (diameter, 382 nm), as would be expected.

Next, it was verified whether the MAb–MNP nanocomposites could be used in the development of the selective filtration. To this end, negative and positive (2×10^4 cells)
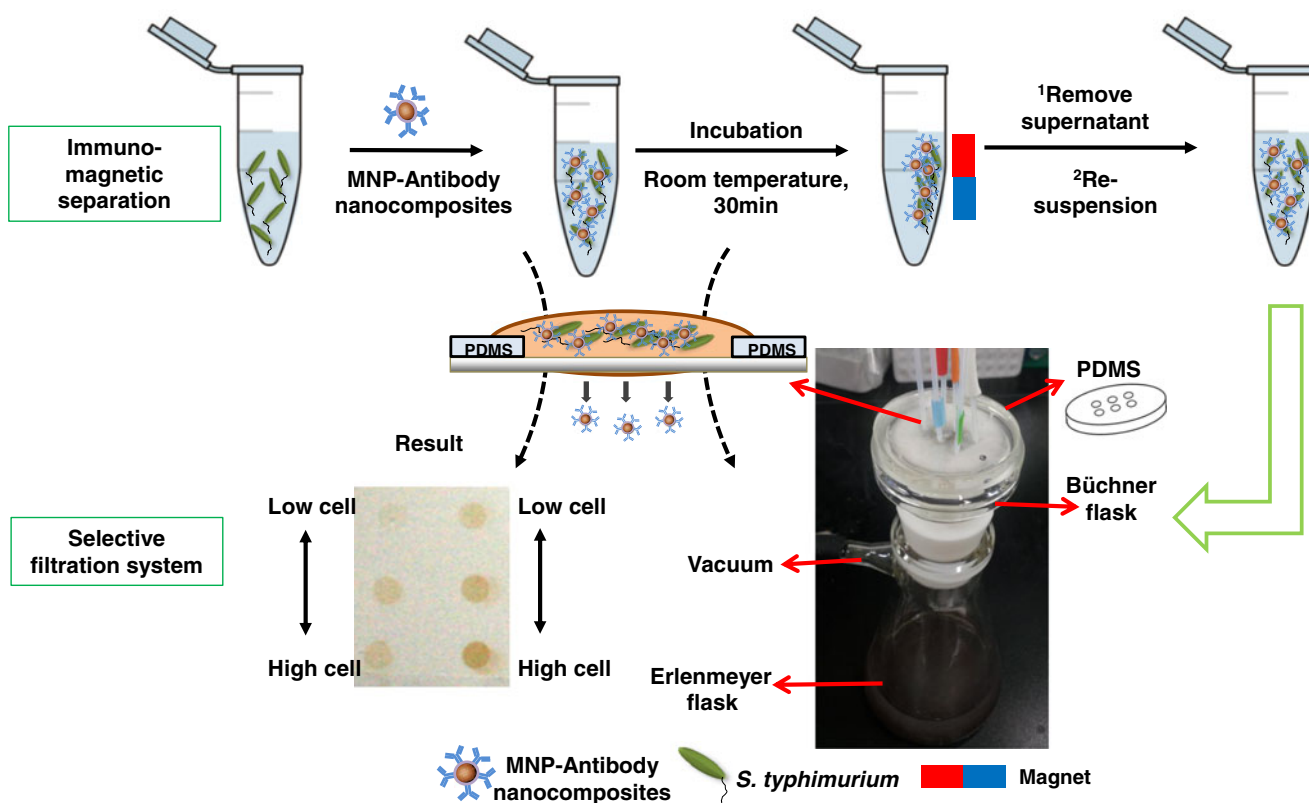
Fig. 1 Schematic diagram of a selective filtration method based on a monoclonal antibody (MAb)–magnetic nanoparticle (MNP) conjugate for the rapid, visible, and ultrasensitive detection of *S. typhimurium*. A diameter of six holes on PDMS film is 3 mm

samples of *S. typhimurium* were respectively mixed with BSA–MNPs or MAb–MNPs and subjected to selective filtration. BSA–MNPs incubated with both positive and negative samples all passed through a nitrocellulose filter membrane (1.2 μm pore size) and did not exhibit any spot on the filter membrane. This means that use of BSA as a blocking reagent on MNPs did not cause non-specific binding to the filter membrane. On the other hand, negative and positive tests with MAb–MNP nanocomposites showed different results on the filter membrane. No signal was observed from the negative test, while clear brown spots were obtained with the positive test. These results verified that the MAb–MNP nanocomposites could be used to develop the selective filtration method.

Capture efficiency of MAb–MNP nanocomposites for the colorimetric detection of *S. typhimurium*

To determine the capture efficiency of MAb–MNP nanocomposites for isolating *S. typhimurium* in PBS, 0.2 mL of the bacteria, ranging in concentration from 2×10^1 to 2×10^4 cells, was mixed with 10 μL of MAb–MNP nanocomposites diluted 1/20, and the mixtures incubated for 30 min at room temperature. The complexes (*S. typhimurium*–MAb–MNP nanocomposites) were isolated, spread onto a XLD agar, and incubated for 24 h at 37 °C. To quantitate capture efficiencies,

the number of captured cells to the initial number of cells used was calculated using the formula:

$$\text{Capture efficiency}(\%) = \left(C_{\text{sep}} / C_{\text{total}}\right) \times 100$$

where C_{total} is the total amount of cells mixed with the MAb–MNP nanocomposites, and C_{sep} is the number of colonies on the XLD agar. As shown in Fig. 2, the capture efficiencies for 2×10^4, 2×10^3, 2×10^2, and 2×10^1 cells were 8, 32, 43, and 90 %, respectively. Because of the limited amount of MAb–MNP nanocomposites, an increasing amount of *S. typhimurium* gradually decreased capture efficiencies. If more MAb–MNP nanocomposites were added to the samples, capture efficiency should be significantly improved compared with that presented here. However, non-specific binding for the negative sample was observed once a higher concentration of MAb–MNP was used. A previous report described a 70 % capture efficiency for 10^2 CFU/mL [17], but the amount of MAb–MNP nanocomposites used greatly exceeded that used in the present study, which optimized cost and efficiency, since the present capture efficiency is sufficient to isolate *S. typhimurium* in the working buffer.
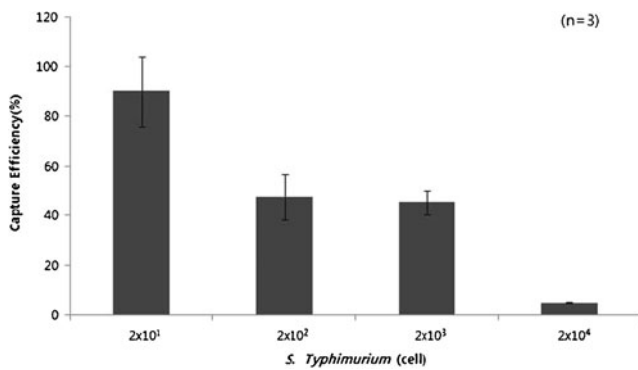
Fig. 2 Capture efficiencies of monoclonal antibody (MAb)–magnetic nanoparticle (MNP) and bovine serum albumin (BSA)–MNP nanocomposites for *S. typhimurium* at 2×10^1, 2×10^2, 2×10^3, and 2×10^4 cells in PBS ($n=3$)

Optimization of the selective filtration system for S. typhimurium

The selective filtration method was optimized by testing some key parameters, such as the appropriate amount of MAb–MNP nanocomposites, various types of membrane, working buffers, and incubation periods and temperature. To determine the appropriate amount of MAb–MNP nanocomposites, undiluted and diluted (1/5, 1/10, 1/20, 1/30, 1/40, 1/50, 1/60, and 1/70 in PBS) MAb–MNP nanocomposites were mixed with *S. typhimurium* negative and positive (2×10^4 cells) samples. Undiluted MAb–MNP nanocomposites and nanocomposites diluted up to 1/10 appeared as a brown spot on the membrane in the presence of both negative and positive tests, indicating non-specific binding, while no signal was determined from negative samples with diluted ($\geq$1/20) MAb–MNP nanocomposites. In contrast, at this dilution, the positive sample appeared as a brown spot on the filter membrane. However, increasing the dilution of the MAb–MNP nanocomposites decreased the color intensities of the spot on the filter membrane in the positive tests. Therefore, we chose to use a 1/20 dilution of MAb–MNP nanocomposites for further use, as it showed the greatest differentiation between the signals of the negative and positive tests.

The filter membrane is the most important factor in the development of selective filtration. To select a suitable membrane, several filter membranes with different pore sizes, ranging from 0.8 to 1.2 μm (nitrocellulose filter membranes with 1.2 μm pores, mixed cellulose ester filter membrane with 1.2 μm pores, MMM filter membrane, and acetate filter membrane with 0.8 μm pores) were chosen in this study. As shown in Fig. 3, the nitrocellulose filter membrane exhibited a clear difference between positive (2×10^4 cells; clear spot) and negative (PBS; no spot) tests. However, other filter membranes produced non-specific binding in negative tests; this included the mixed cellulose ester membrane possessing the same pore size as the nitrocellulose filter membrane (1.2 μm).

The pore size of the filter membrane greatly varies, with multiple layers of pores stacked on top of one another to form a tortuous flow path. Although several filter membranes ensure the capture of particles bigger than the specified pore size, it could also lead unexpected retention of small particles, such as nanocomposites not bound to *S. typhimurium*, by trapping them in the pores of the filter membrane and absorbing them into the layers of the membrane. Therefore, although membranes possess the same pore size, their performance on the selective filtration could differ due to differences in their inner structures. We thus selected the nitrocellulose filter membrane with a 1.2 μm pore size for further investigation.

Additionally, various solutions, such as PBS (pH 7.2), PBST (pH 7.2), tryptic soy broth, 0.85 % NaCl, 0.1 M Tris–HCl (pH 7.4), and 20 mM borate buffer (pH 7.4) were tested as working buffers for the selective filtration. Most of these caused non-specific binding in the negative test, but no non-specific binding was obtained when PBS was used (Fig. S2, Electronic supplementary material). Consequentially, PBS was chosen as a suitable working buffer for the development of the selective filtration method.

Finally, the incubation period for the mixture of MAb–MNP nanocomposites and *S. typhimurium* was optimized. The incubation was carried out for 10, 20, and 30 min at 4, 25, and 37 °C, respectively, and the samples then subjected to the selective filtration. Increasing the incubation time gradually increased the color intensities of the brown spot for positive samples (2×10^1 to 2×10^4 cells). The color intensities increased linearly with the numbers of *S. typhimurium* after 20 and 30 min incubation. Incubation for 30 min was chosen as an optimum period, but we did not investigate longer incubation time due, as this was the least amount of time that was sufficient to allow distinction of color intensities between positive and negative tests. Similarly, the effect of the incubation temperature on selective filtration was investigated. Room temperature (25 °C) was selected for the incubation of MAb–MNP nanocomposites and samples because it exhibited a good linearity, corresponding to the numbers of *S. typhimurium* from 2×10^1 to 2×10^4 cells, and eliminates the need for an incubator or refrigerator to adjust the temperature (Fig. S3, Electronic supplementary material).

Validation of the selective filtration system

The selective filtration method was validated by determining sensitivity with various concentrations of *S. typhimurium* ranging from 0 to 2×10^4 cells, as well as cross-reactivity to other bacteria and by analysis of vegetable samples artificially inoculated with *S. typhimurium*. Figure 4 shows the intensities of the brown spots produced by target cell–MAb–MNP nanocomposites on the filter membrane; these intensities were converted to numeric values to produce a typical standard curve (Fig. 4, bottom). No brown spot was obtained from

Fig. 3 Selection of an appropriate membrane for the selective filtration method. **a** Nitrocellulose filter membrane (1.2 μm pore size); **b** mixed cellulose ester filter membrane (1.2 μm); **c** acetate cellulose filter membrane (0.8 μm); **d** MMM filter membrane (0.8 μm); *N* negative test (PBS); *P* positive test (2×10^4 cells in PBS) (*n*=3)
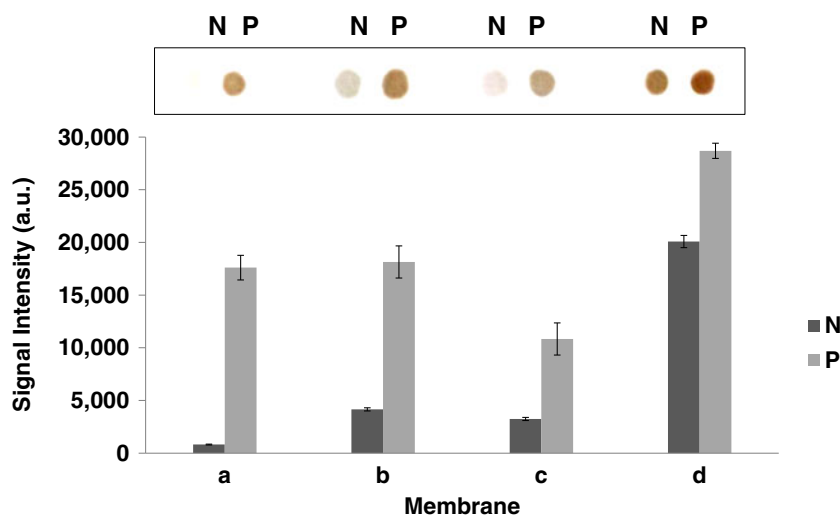
the negative test, and the color intensity gradually increased with increasing amounts of target bacteria. The regression equation of the standard curve was $y = 3,657x - 1,479.1$, with $R^2 = 0.99$. According to the equation, the detection limit of the method, which is the lowest bacterial count with a color intensity at least three times higher than the standard deviation of the negative test, was 3 cells.

In addition, the color intensities between 0 and 2×10^1 cells were clearly different, and the distinction of numeric values converted for concentrations between 0 and 2×10^1 cells was more than 4,000 signal intensities. This indicates that the method developed in this study can be used for visible and qualitative detection of *S. typhimurium*. As a visible and qualitative method, the selective filtration method is highly sensitive (visible detection limit, 2×10^1 cells). To our knowledge, no visible method exhibiting better sensitivity has been reported. As shown in Table 1, there are many analytical methods combined with MNP or magnetic beads and immuno-nanoparticles conjugated with recognition elements and used as capture reagents for *S. typhimurium* diagnosis. Preechakasedki et al. [19] developed an immunochromatographic strip test possessing the limit of detection (LOD) of 1.14×10^5 cfu/ml for *S. typhimurium* that does not require any equipment and takes 15 min to yield results. Ko and Grant [8] reported a fluorescence resonance energy transfer (FRET) for the rapid and simple detection of *S. typhimurium*. They emphasized that the method can finished in 10 min with one experiment step, but the method possesses higher LOD compared with the present method and requires an equipment to get results. Joo et al. [1] used antibody-immobilized MNPs and TiO2 nanocrystals coupled with another antibody to develop an optical sensor for *S. typhimurium* detection. The LOD of the method was 10^2 CFU/mL. Wen et al. [15] developed the coupling fluorescent sensor, employing immunomagnetic nanospheres and immunofluorescent nanospheres, to detect *S. typhimurium* with a LOD of 10 CFU/mL

in a pure sample. An electrochemical sensor for *S. typhimurium* diagnosis was developed and applied to skimmed milk samples. This method can also detect 10 CFU/mL of *S. typhimurium* [21]. On the other hand, an electrochemical magneto genosensor was developed by Liébana et al. [18] in order to detect 1 CFU in skim milk for *S. typhimurium*. However, the method still requires a PCR machine to produce results and takes 3.5 h to complete the analysis. As mentioned above, although many analytical methods have been recently developed for *S. typhimurium* and applied to real samples, most of them still a special equipment to detect fluorescence and electric signals. The sensitivity of the method developed in the present study is comparable to those of these methods mentioned above. Moreover, MNPs or magnetic beads used in other studies were only exploited as a capture reagent, but the MAb–MNPs used in this study were employed as detector reagent. Whereas the proposed method requires fewer
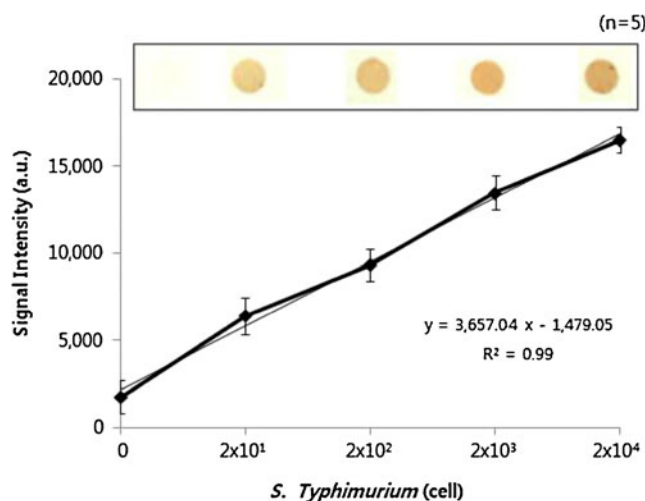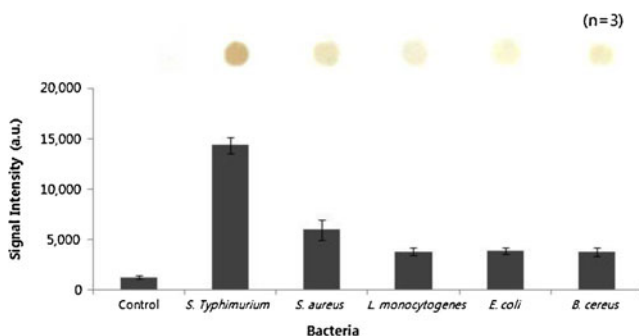

Fig. 4 Linear relationship of the intensities of the colored spots on the filter membrane versus bacterial concentrations from 0 to 2×10^4 cells in PBS

Table 1 Comparison of the method developed in this study with other methods for *S. typhimurium* analysis

| Detection method	Samples	Limit of detection	Time	Equipments required	References
Coupling magnetic capture and fluorescence nanosphere	Milk, fetal bovine serum, urine	10 cfu/ml	>1 h	Confocal microscope	[15]
Fluorescence resonance energy transfer (FRET)	Pork	10^5 cfu/g	>10 min	Benchtop fluorometer	[8]
Electrochemical magneto genosensor	Skim milk	1 cfu/ml	<3.5 h	PCR machine	[18]
Immunochromatographic strip test	Human serum	1.14×10^5 cfu/ml	15 min	–	[19]
Magnetic nanoparticles and optical nanocrystal probes	Milk	10^2 cfu/ml	40 min	UV–vis spectrometer	[1]
A bead-free quantum dot-facilitated isolation method	Ground beef	10 cfu/g	>2.5 h	FluoroMax-3 spectrophotometer	[20]
Magneto-immunoassay and gold nanoparticles	Skim milk	1.43×10^2 cfu/ml	>1.5 h	Electrochemical analyzer Autolab 20	[21]
Selective filtration method	Lettuce and cabbage	100 cells/g	<45 min	–	This work

experimental steps and can be completed within 45 min (incubation, 30 min; washing, 10 min; filtration, 5 min). Compared with other sensors mentioned above the selective filtration method has several advantages, such as being simple, easy-to-use, sensitive, low-cost, without requiring expensive instruments and dissociation steps of target bacteria from magnetic beads, and rapidly yielding colorimetric results; these features make it an attractive tool for simple, sensitive, and rapid *S. typhimurium* diagnosis (Table 1). In addition, the filter membrane used in this study can be easily burned, and this feature has a significant meaning at preserving the environment when the method is applied to point-of-care testing.

The specificity of the method presented here was also evaluated with untargeted bacteria (2×10^4 cells) such as *S. aureus*, *L. monocytogenes*, *E. coli*, and *B. cereus*. As shown in Fig. 5, light brown spots were observed when selective filtration method was performed with these other bacteria. These phenomena can be explained in that some bigger bacteria, such as *E. coli* (0.5 μm in width and 2 μm in length) and *B. cereus* (3–4 μm) can block the pores of the filter, resulting in the deposition of MAb–MNP nanocomposites on the bigger bacteria blocking the pore. Moreover, *S. aureus* often

expressed protein A, which possesses affinity to IgG antibodies, which is the type of MAb used in this study. Although a light brown spot was obtained with these other bacteria, the color intensities were much lower than the intensity obtained with *S. typhimurium* (2×10^1 cells). Therefore, the method in the present study was regarded to be specific to *S. typhimurium* and can be used for the visible detection of *S. typhimurium*.

Fig. 5 Specificity study for the selective filtration performed in PBS with *S. aureus*, *L. monocytogenes*, *E. coli*, and *B. cereus* at 2×10^4 cells and *S. typhimurium* at 2×10^1 cells ($n = 3$)
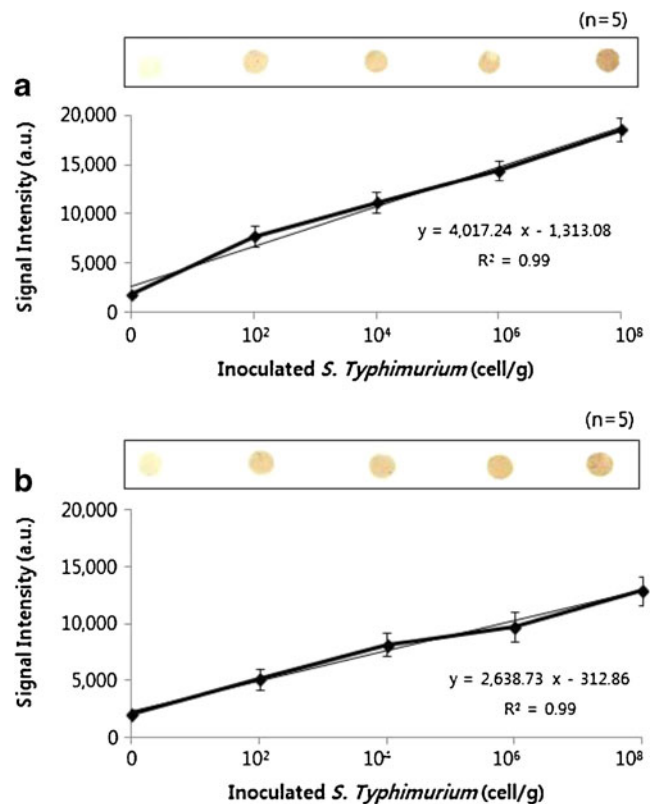
Fig. 6 Linear relationship of the intensities of colored spots on the filter versus inoculated bacteria concentrations from 0 to 10^8 cells/g in vegetables. **a** Lettuce; **b** cabbage

Performance in vegetable samples artificially inoculated with *S. typhimurium*

Figure 6 shows the result of analysis of cabbage and lettuce samples artificially inoculated with *S. typhimurium* at 0, 10^2, 10^4, 10^6, and 10^8 cells/g which will be 0, 2×10^1, 2×10^3, 2×10^5, and 2×10^7 cells of sample solutions after sample preparation. Even though light brown spots were obtained from cabbage and lettuce samples that had not been inoculated, these spots could be regarded as negative tests since their intensities were markedly lower than those obtained from *S. typhimurium*-positive samples. On the other hand, the color intensities of these brown spots gradually increased with an increase in the pathogen inoculated in the vegetables. The linear section of the standard curve had a regression equation of $y = 4{,}017.2x - 1313.1$ with $R^2 = 0.99$ for lettuce and of $y = 2{,}638.7x - 312.9$ with $R^2 = 0.99$ for cabbage.

The visible detection limit for *S. typhimurium* inoculated in either type of vegetable was 100 cells/g. In addition, the total intensity of the brown spot on the filter membrane derived from these samples was slightly less than that obtained with pure bacteria in PBS. This is probably because various substances co-extracted from the vegetable samples could lead to inhibition of the interaction between MAb–MNPs and *S. typhimurium*. However, as shown in Fig. 6, the selective filtration method not only detected 100 cells/g of *S. typhimurium* in both vegetables, but also yielded a visible result within 45 min when applied to real samples.

Conclusions

A rapid, facile, and ultrasensitive selective filtration method based on MAb–MNP nanocomposites was developed for the colorimetric detection of *S. typhimurium* in vegetable samples. The MAb–MNP nanocomposites in pure bacterial samples or sample extracts efficiently bound to *S. typhimurium* and were isolated magnetically. Consequentially, sufficient capture efficiencies for various concentrations of the pathogen were determined. After optimization, the developed selective filtration method was able to visibly detect up to 2×10^1 cells or 100 cells/g in pure pathogen solution and vegetables, respectively. The method was also confirmed to be specific for *S. typhimurium* and required a shorter or similar assay time (45 min) to other methods based on antibody-immobilized MNPs that have recently been reported. The present study demonstrated that MAb–MNP nanocomposites can be independently used as a good detector reagent, without the need for pairing these particles with antibody-immobilized nanoparticles such as gold and fluorescent nanoparticles or nanocrystals to constitute a sandwich assay. This study described a simple and convenient method to synthesize MAb–MNP nanocomposites and demonstrated its direct application

without a dissociation step of bacteria from magnetic beads in colorimetric assays in practice. The selective filtration method also offers many advantages, such as being simple, ultrasensitive, easy to perform, and allowing rapid detection of pathogenic bacteria. The method could be concluded within 45 min, yielding results in a shorter or similar time period as compared with recently reported antibody immobilized on magnetic particle-based methods.

References

1. Joo J, Yim C, Kwon D, Lee J, Shin HH, Cha HJ, Jeon S (2012) Analyst 137:3609–3612
2. WHO (2011) Food safety and food borne illness. <http://www.who.int/mediacentre/factsheets/fs237/en/>
3. Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, Opsteegh M, Langelaar M, Threfall J, Scheutz F, van der Giessen J, Kruse H (2010) Int J Food Microbiol 139:S3–S15
4. Crump JA, Mintz ED (2010) Clin Infect Dis 50:241–246
5. Crump JA, Luby SP, Mintz ED (2004) Bull World Health Organ 82:346–353
6. Singh G, Vajpayee P, Rani N, Jyoti A, Gupta KC, Shanker R (2012) Ecotoxicol Environ Safe 78:320–326
7. Liu Y, Che Y, Li Y (2001) Sensors Actuat B 72:214–218
8. Ko S, Grant SA (2006) Biosens Bioelectron 21:1283–1290
9. Yang ZY, Shim WB, Kim KY, Chung DH (2010) J Agric Food Chem 58:7135–7140
10. Jadeja R, Janes ME, Simonson JG (2010) J Food Protect 73:1288–1293
11. De Lamo-Castellvi S, Manning A, Rodriguez-Saona LE (2010) Analyst 135:2987–2992
12. Shin J, Kim M (2008) J Microbiol Biotechnol 18:1689–1694
13. Shim WB, Choi JG, Kim JY, Yang ZY, Lee KH, Kim MG, Ha SD, Kim KS, Kim KY, Kim CH, Eremin SA, Chung DH (2008) J Food Protect 71:781–789
14. Guven B, Basaran-Akgul N, Temur E, Tamer U, Boyaci IH (2011) Analyst 136:740–748
15. Wen CY, Hu J, Zhang ZL, Tian ZQ, Ou GP, Liao YL, Li Y, Xie M, Sun ZY, Pang DW (2013) Anal Chem 85:1223–1230
16. Sung YJ, Suk HJ, Sung HY, Li T, Poo H, Kim MG (2013) Biosens Bioelectron 43:432–439
17. Yang Y, Xu F, Xu H, Aguilar ZP, Niu R, Yuan Y, Sun J, You X, Lai W, Xiong Y, Wan C, Wei H (2013) Food Microbiol 34:418–424
18. Liébana S, Lermo A, Compoy S, Barbé J, Alegret S, Pividori MI (2009) Anal Chem 81:5812–5820
19. Preechakasedkit P, Pinwattana K, Dungchai W, Siangproh W, Chaicumpa W, Tongtawe P, Chailapakul O (2012) Biosens Bioelectron 31:562–566
20. Wang L, Wu CS, Fan X, Mustapha A (2012) Int J Food Microbiol 156:83–87
21. Afonso AS, Perez-Lopez B, Faria RC, Mattoso LH, Hernandez-Herrero M, Roig-Sagues AX, Maltez-da Costa M, Merkoci A (2013) Biosens Bioelectron 40:121–126