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Rapid detection of *Listeria monocytogenes* in food by biofunctionalized magnetic nanoparticle based on nuclear magnetic resonance

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ABSTRACT

In order to ensure the safety of food, particularly ready-to-eat (RTE) food, a sensitive and rapid method for the detection of foodborne pathogenic bacteria is always in high demand. An accurate and reliable detection method for *Listeria monocytogenes* has been developed based on the aggregation of biofunctionalized magnetic nanoparticles induced by specific binding between *L. monocytogenes* and antibody-modified nanoparticles, which results in the change of the transverse relaxation time (T2) of surrounding water protons of nanoparticles detected by nuclear magnetic resonance. The detection limit of this method is 3 MPN (using the most-probable-number (MPN) assay) and the functionalized Fe/Fe_3O_4 nanoparticles exhibit a high specificity with the existence of other interfering bacteria. Furthermore, this detection method is successfully applied to detect *L. monocytogenes* in milk powder and lettuce. This method could be a useful tool for sensitive and rapid detection of foodborne pathogenic bacteria.

1. Introduction

Listeria monocytogenes is an important foodborne pathogen which is found in raw and processed foods. Currently there is an increasing demand for ready-to-eat (RTE) foods but RTE foods can be sources of *Listeria* infections (Välimaa, Tilsala-Timisjärvi, & Virtanen, 2015). The genus *Listeria* includes seven species (Doijad et al. 2011), of which only *L. monocytogenes* and *Listeria ivanovii* are pathogenic. Notably, *L. monocytogenes* is a major Gram-positive foodborne pathogen in both human beings and animals, which has already raised great concerns in several countries. It causes meningitis, neonatal listeriosis, septicemia and abortion on infected humans (McLauchlin, Mitchell, Smerdon, & Jewell, 2004). The occurrence of listeriosis is quite low but it has high hospitalization rate (94%) and a high case-fatality rate (12.8–17% of the cases) (Gilliss et al. 2011; Melo, Andrew, & Faleiro 2015). The conventional detection method of *L. monocytogenes* are is simple and costs little, but involves multiple time-consuming and labor-intensive steps (D'Amico & Donnelly, 2009; Jayarao & Henning, 2001; Van Kessel, Karns, Gorski, McCluskey, & Perdue, 2004). In addition to traditional methods, there are some rapid detection methods for *L. monocytogenes* (Jadhav, Bhave, & Palombo 2012; Liu, 2006), such as enzyme-linked fluorescent assay (ELFA) (Jaakohuhta, Härmä, Tuomola, & Lövgren, 2007), the multiplex polymerase chain reaction (PCR) assay (Amagliani, Giammarini, Omiccioli, Brandi, & Magnani, 2007), isothermal amplification (Cho, Dong, Seo, & Cho, 2014; Wan et al. 2012), and Biosensor-based techniques (Davis et al. 2013; Radhakrishnan, Jahne, Rogers, & Suni, 2013). These methods can achieve high specificity and low minimum detection limit for the detection of *L. monocytogenes*; however, they are very expensive and some of them may still produce false-positive results (Välimaa et al. 2015). Therefore, the development of fast and sensitive detection approaches enabling the identification of low levels

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of the foodborne pathogens is necessary.

In recent years, biomolecule detection methods with nuclear magnetic resonance (NMR) as the underlying principle have developed rapidly and been applied widely. The magnetic relaxation switch (MRS) is based on magnetic nanoparticles (MNPs) having ability to switch between a dispersed and assembled state upon analyte binding on the surface of nanoparticles (NPs) by low field NMR, with a concomitant change in the relaxation time of the solution's water protons (Kaittanis, Santra, & Perez 2009; Koh & Josephson, 2009; Shao et al. 2012; Taktak, Sosnovik, Cima, Weissleder, & Josephson, 2007). This has been designed to selectively detect a variety of metal ions (Atanasijevic, Shusteff, Fam, & Jasanoff, 2006; Zhang et al. 2014) and small molecular and biological targets including nucleic acids (Josephson, Manuel Perez, & Weissleder 2001; Perez, O'Loughin, Simeone, Weissleder, & Josephson, 2002), proteins (Cai et al. 2011; Kulkarni, Weiss, & Iyer 2010), algae toxins (Ma et al. 2009), viruses (Perez, Simeone, Saeki, Josephson, & Weissleder, 2003), bacteria (Kaittanis, Naser, & Perez, 2007; Zhao et al. 2013) and enantiomeric impurities (Tsourkas, Hofstetter, Hofstetter, Weissleder, & Josephson, 2004). For example, a sensitive NMR-based detection method has been established to identify *Bacillus Calmette-Guerin* (BCG) and demonstrated that the detection limit reached as few as eight bacterial cells per mL in experimental samples within 1 h (Liang, Chen, Zhang, Wu, & Kong, 2012); A rapid and simple immune-sensor MRS assay has been developed for the detection of Kanamycin (KM) in milk with limit of detection (LOD) of 0.1 ng mL^{-1} (Chen et al. 2013). In addition, Kaittanis et al., (2007) established a fast bacterial detection method based on superparamagnetic iron oxide nanoparticles to identify *Mycobacterium avium* spp. *paratuberculosis* (MAP) in milk and blood through magnetic relaxation (Kaittanis et al. 2007).

In this paper, we demonstrate a rapid method of detecting *L. monocytogenes* in food which is based on the magnetic relaxation of magnetic Fe/Fe_3O_4 nanoparticles (Fe/Fe_3O_4 NPs). Fe/Fe_3O_4 NPs consisting of an elemental Fe-core and a protective ferrite shell showing high stability against oxidation and strong magnetization (Castro et al. 2014). Fe/Fe_3O_4 NPs were modified with silica and coupled with anti- *L. monocytogenes* antibodies. We found that dispersed nanoparticles in solution self-assemble on the surface of a bacterial target accompanied by the increase of the T2 value of water protons. We can determine the presence of *L. monocytogenes* on the basis of the change of T2 value (ΔT2) using NMR. Therefore, this method is a potential tool for the rapid detection of food-borne bacteria.

2. Materials and methods

2.1. Reagents

Listeria monocytogenes Antisera (O VII) were purchased from Denka Seiken (Tokyo, Japan). 1-[3-(dimethylamino)-propy-3-ethylcarbodiimide HCL (EDC HCL) and *N*-hydroxy-succinimide (NHS)] were purchased from SinopHarm (Shanghai, China). Nutrient broth medium and other media were obtained from Beijing Land Bridge Technology Co. Ltd. (Beijing, China). Chemical reagents and salts were obtained from Shanghai Ling Feng Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Apparatus

Transmission electron microscopy (TEM) was carried out with a JEOL JEM-2010 transmission electron microscope at 200 kV. X-ray diffraction (XRD) was performed using a Rigaku DMAX 2000 diffractometer equipped with Cu/K radiation at a scanning rate of 4°/min in the 2 range from 10 to 80° (= 0.15405 nm, 40 kV, 40 mA). FT-IR spectra were collected on a Nicolet Avatar 370. The samples were pelletized with KBr before measurements. T2 value was measured on a 0.5T NMI 20-CA (Shanghai Niumag Corporation Limited).

2.3. Synthesis of Fe/Fe_3O_4@SiO_2-NH_2NPs

Fe/Fe_3O_4 NPs were prepared by the pyrolysis method as described previously via the precursor of iron pentacarbonyl in the presence of hexadecylammonium chloride at 180 °C in the mixture of octadecene and oleylamine under the protection of nitrogen (Lacroix et al. 2011; Shen et al. 2014). In order to empower Fe/Fe_3O_4 NPs with stability and biocompatibility, NPs were functionalized. Fe/Fe_3O_4 NPs were dispersed in 60 mL cyclohexane and Triton-X100 (1.1 mL) with sonication (45 min) until the mixture dispersed evenly. Then ammonium hydroxide (0.15 mL) and 1-octanol (dropping until the mixture solution became clear) were added to the above solution with continuous stirring. 0.16 mL TEOS and 0.04 mL APS were added and the reaction was continued for 72 h at room temperature. The final product was precipitated with acetone and centrifuged with ethanol.

2.4. Coupling the antibodies to the magnetic beads

According to a previous report (Zhao et al., 2013), 1-p-3-ethylcarbodiimide HCL (EDC·HCL) and *N*-hydroxy-succinimide (NHS) were used to activate the carboxyl of the antibody. 200 mg EDC·HCL and 250 mg NHS were added to the 0.1 mL *Listeria monocytogenes* antibody. Then the mixture was diluted into 5 mL PBS (pH 7.4). After activation for 60 min, amino-modified silica-coated Fe/Fe_3O_4 was dispersed in 5 mL PBS (pH 7.4). Then the upper mixture was added to the activated antibody solution and left to react for 4 h at room temperature. Finally, excess antibodies were removed by centrifugation and washed by PBS (pH 7.4) three times. The final product was re-dispersed in PBS (pH 7.4) at 4 °C.

2.5. Bacterial culture

Listeria monocytogenes (ATCCA 19114) was used throughout this study for all detection and sensitivity experiments. In addition, *Escherichia coli O157* (ATCC 8089), *Salmonella typhimurium* (ATCC 14028), *Cronobacter sakazakii* (ATCC29544) *Staphylococcus aureus* (ATCC 6538), *Listeria innocua* (ATCC 33090), *Listeria iuanuii* (ATCC 19119) and *Listeria seeligeri* (ATCC35917) were used in competition experiments to determine the specificity of the biofunctionized NPs. All control strains were selected and cultured separately in relevant chromogenic medium, and then in nutrient broth. *Listeria* strains were cultured in *Listeria* enrichment broth. All of the bacteria were incubated at 36 ± 1 °C (*Listeria* for 30 °C) for 18–24 h in an orbital shaker. The bacteria number of each pure strain of *Listeria monocytogenes*, *Escherichia coil O157*, *C. sakazakii*, *S. typhimurium* and *S. aureus* was determined by the aerobic plate count method with plate count agar. 0.5% formaldehyde was then added to the bacteria samples for safety and controlling the number of bacteria.

The number of bacteria less than 30 was determined by the most-probable-number (MPN) assay. Based on the growth observed at higher dilutions, the MPN of survivors was calculated using a "nine-tube" technique according to China national food safety standard GB 4789.30 and GB4789.2. However, we used flat colony counting method when the number of bacteria was more than 30. The lowest bacteria concentration detected by MPN was 3.