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Rapid detection of food-borne *Salmonella* contamination using IMBs-qPCR method based on *pagC* gene

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ABSTRACT

Detection of *Salmonella* is very important to minimize the food safety risk. In this study, the recombinant PagC protein and PagC antibody were prepared and coupled with immunomagnetic beads (IMBs) to capture *Salmonella* cells from pork and milk samples. And then the SYBR Green qualitative PCR was developed to detect the pathogenic *Salmonella*. The results showed that the PagC polyclonal antiserum is of good specificity and the capture rate of 0.1 mg IMBs for *Salmonella* tended to be stable at the range of 70–74% corresponding to the concentrations between 10^1 and 10^4 CFU/mL. The method developed demonstrated high specificity for the positive *Salmonella* samples when compared to non-specific DNA samples, such as *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*. The limit of detection of this assay was 18 CFU/mL. Detection and quantitative enumeration of *Salmonella* in samples of pork or milk shows good recoveries of 54.34% and 52.07%. In conclusion, the polyclonal antibody of recombinant PagC protein is effective to capture *Salmonella* from detected samples. The developed pagC antibody IMBs-qPCR method showed efficiency, sensitivity and specificity for 30 *Salmonella* detection, enabling detection within 10 h, which is a promising rapid method to detect *Salmonella* in emergency.

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Introduction

Salmonella species have been found to be responsible for food-borne illnesses such as typhoid and paratyphoid fevers, gastroenteritis, pneumonia and bacteraemia. The major sources of salmonellosis outbreaks include meat, poultry, fish/seafood, eggs and processed food.[1,2] Food-borne salmonellosis is a huge public health burden not only in the developing countries but also in industrialized countries, leading to high rates of enteric diseases, hospitalizations and even deaths worldwide each year.[3–5] As one of the most important food borne pathogens, *Salmonella* infects over 160,000 individuals in the EU annually, with a morbidity rate of 35 cases per 100,000.[6] *Salmonella* was the second laboratory-confirmed etiologic agent account for 229 (30%) reported food poisoning outbreaks in the United States[7] and the annual economic cost of *Salmonella* infections is at $2.4 billion.[8] In Hong Kong, *Salmonella* spp. account for 22% of foodborne disease outbreaks during 2000–2004.[9]

Detection of pathogenic *Salmonella* in various food matrixes is very important to minimize the food safety risk. Conventional culture detection methods are time-consuming and labour-intensive, as it takes 3–7 days including selective enrichment steps for positive results, which is inconvenient for rapid detection in food industry. Immunology-based methods involving antigen–antibody bindings have been widely used for the detection of food-borne pathogens. Immunomagnetic bead separation (IMS) techniques use antibodies against the outer membrane protein (OMP) of *Salmonella* attached to a magnetic microbeads. These immunomagnetic beads can combine and concentrate *Salmonella* bacteria in detection samples and then be used in subsequent detection assay, such as the technique of real-time quantitative PCR (qPCR), ELISA, fluorescence immunoassay and flow cytometry (FCM),[10–14] which may improve the efficiency and sensitivity, and decrease the non-specificity.[15–18]

PagC protein expressed in most pathogenic *Salmonella* is a 188-aminoacid outer membrane protein coded in chromosome of *Salmonella* which is known to be with strong immunogenicities and immunoprotection.[19,20] PagC is a protein regulated by phoP-phoQ involved in bacterial virulence and macrophage survival of *Salmonella typhimurium*,[21] and played an essential role in the serum resistance of *S. enterica serovar* choleraesuis.[22,23] Furthermore, the prevalence of pagC was significantly higher ($p < 0.01$) among the isolates from the diseased pigs than in isolates from the healthy pigs.[22] The expression of PagC is activated by conditions that mimic acidified macrophage phagosomes.[24] The recombinant PagC outer membrane protein is a promising tool for the serum resistance upon *Salmonella*.[20]

It has been shown that real-time PCR methods are very effective to detect *Salmonella* organisms after pre-enrichment of foods.[25] To avoid the false-positive results from the detection of dead cells, a TaqMan quantitative real-time RT-PCR (qRT-PCR) assay to assay the invA mRNA level of *Salmonella* was developed.[26] However, the invA mRNA levels vary with temperature, manure or culture conditions.[27] Different from the monoclonal antibody against *Salmonella* somatic antigen, the polyclonal antibodies of recombinant OMP antigen keep the strong bacterial recognition ability, good antigen-antibody reactivity and multi-pathogenic isolate compatibility, but a lower cross-reactivity as polyclonal antibody against whole bacterial antigen do. In our study, an alternative rapid method combining SYBR Green qPCR and immunomagnetic beads coated with specific PagC antibody was developed to quantify *Salmonella* cells in complex food matrix contaminated.

Methods

Bacterial strains and culture conditions

The bacterial strains and plasmids used in this study are described in Table 1. All *Yersinia* strains were cultured in Luria–Bertani (LB) broth (DingGuo, Beijing, China) at 28 °C for 14 h, and other strains were incubated at 37 °C in LB with gentle shaking. All of the bacterial strains were supplemented with glycerol (final concentration of 25%) and stored in a refrigerator at -80 °C until use.

Expression and purification of recombinant PagC protein

The gene *pagC* was PCR-amplified from the DNA of *Salmonella* using primers harbouring restriction sites (underlined): *pagC*-F (5′-CGGGATCCAGCGTTT TGGTTGTAAATG-3′) and *pagC*-R (5′-CCGCTCGAGTCAGA AACGGTATCCAAYT-3′). The PCR conditions initial denaturation at 94 °C for 6 min followed by the 5 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 35 s and then 25 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 35 s; finally, extension at 72 °C for 6 min. The 1% agarose gel with ethidium bromide (0.5 μg/mL) was used to analyze the amplicons. The PCR product was cloned into the TA cloning vector pUC-T Simple (CWBIO Corp, Beijing, China) to generate pUC-*pagC*. The BamHI–XhoI fragments of pUC-*pagC* were ligated with the same sites of pET28a to produce pET28a-*pagC*. Plasmid pET28a-*pagC* in *Escherichia coli* Top10 (TransGen Biotech, Beijing, China) producing recombinant PagC was sequenced (Genewiz Corp., Beijing, China) and no frame shift or other mutations in the coding sequence of PagC were detected. Expression and purification of recombinant PagC were performed by using procedures described as our previously report.[28] Equal amounts of protein were subjected to SDS-PAGE in 12% polyacrylamide gel with Mini-Protean (BioRad) apparatus as previously described.[29] Protein concentrations were determined by using Bicinchoninic acid (BCA) protein assay, with BSA (Solarbio Corp, Beijing, China) as a standard.

Generation of polyclonal antisera of recombinant PagC protein

Approximately 1 mg of highly purified PagC obtained from *E. coli* BL21(DE3) (TransGen Biotech, Beijing, China) was used for production of polyclonal antisera. The SPF Rabbit purchased from Tianjin Laboratory Animal Center were maintained in SPF (Specific pathogen-free) facilities and was immunized subcutaneously following standard immunization protocols with 1 mg of recombinant protein emulsified with equal volume of

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