Rapid Detection of *Salmonella enteritidis* by PCR Amplification of The SefA Gene and It's Cloning

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**Abstract:** The emergence of *Salmonella enteritidis* as an important food-borne pathogenesis in humans, demands the development of novel detection and intervention strategies. It is generally accepted that fimbriae are an important factor in bacterial survival and persistence in the host. This study is directed towards the method of amplifying and cloning the SefA gene, which encode *Salmonella enteritidis* fimbral protein. Strains used for these studies were *S. enteritidis* (E3), which were collected from Kermanshah region. Chromosomal DNA was extracted by boiling method and PCR reaction was performed and single band of 511 bp amplified by SefA-F and SefA-R primers. The resulting PCR product was inserted into the cloning vector (pTZ57R/T). In order to amplify the recombinant plasmid, *E. coli* DH5α bacteria were transformed with SefA-pTZ57R/T. Recombinant clones were identified by blue/white selection and purified recombinant plasmids were indicated by an alkaline lysis procedure. Identity of the SefA-pTZ57R/T product was confirmed by RFLP and sequencing. Nucleotide and protein alignment with BLAST software showed that the sequence of the SefA gene derived from *S. enteritidis* (E3), which was cloned in the pTZ57R/T vector, was 99% identical to that of the Genbank (L11008). The sequence of the SefA gene from *S. enteritidis* (E3) differed only in two nucleotides and one amino acid. The cloned SefA gene from *S. enteritidis* (E3) was submitted to the NCBI Genbank (EF553334).

**Key words:** *Salmonella enteritidis*, PCR, cloning, bacterial survival

**INTRODUCTION**

Microbial diseases constitute a major cause of death in many parts of the world. *Salmonella* are considered major health problem worldwide. Salmonella has been identified as an important food and water-borne pathogen (Laconcha et al., 1998; Wilks et al., 2000). *S. enteritidis* emerged as an important pathogen in poultry and human (Kisieila et al., 2003). Human *S. enteritidis* infections showed a dramatic increase since 1980s, particularly in developing countries and has become the most commonly isolated serotype in many countries (Sakai and Chalamjekhat, 1996; Rabsch et al., 2001; Wilks et al., 2000). Recently it has been identified that poultry, consumption of raw eggs, poultry meat, processed products, fast food and international food trade between countries are the major sources of infection and transmission of *S. enteritidis* in humans (Nayak et al., 2004; Wilks et al., 2000; Landeras et al., 1998; Gibson, 2000).

Fimbriae, also called pili, are surface appendage and filamentous structures which are primarily composed of low molecular weight protein (14 kDa), *Salmonella enterica* subsp. *Enterica serovar enteritidis* elaborates morphologically distinct fimbriae designated SeF21, Sef17, Sef1-4 and probably an additional kinds of LPF and PEF (Rajashekara et al., 2000). It is generally accepted that fimbriae are an important factor in bacterial survival and persistence in the host. Fimbriae play an important role in adhesion of bacteria to different cell surfaces. It's known that adhesion of *S. enteritidis* to cell surface is an essential stage in colonization and pathogenesis of salmonellosis (Rajashekara et al., 2000; Ogunkiyi et al., 1997; Darwin and Miller, 1999; Kisieila et al., 2003).

It has been shown that the *S. enteritidis* fimbriae Sef14 are composed of major fimbrial protein called SefA, which are coded by SefA gene (Clouthier et al., 1993; Collinson et al., 1996; Muller et al., 1991). Because of their structure and localization, fimbriae are excellent targets for the immunological system of the host and highly immunogenic (Kisieila et al., 2003; Kuczkowski et al., 2004). *S. enteritidis* fimbriae antigen are useful as an antigen for immunocassay diagnosis of *S. enteritidis* infection or evidence and treatment of infection (Kisieila et al., 2003; Kuczkowski et al., 2004; Oehoa-Reparaz et al., 2004).

**MATERIALS AND METHODS**

**Bacterial strains, plasmid, media and growth conditions:** *S. enteritidis* E3 was provided by S. Ghazaey

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(Mirmomen et al., 2007). E. coli DH5α was used as the host for the cloning experiments with pTZ57R/T vector. Stock culture were maintained at -70°C in 20% (v/v) glycerol. All cultures were routinely grown on agar at 37°C. Cells for DNA extraction were grown in Luria broth/LB at 37°C.

Preparation of genomic and plasmid DNA: Chromosomal DNA was isolated by boiling method. In this method one bacteria colony from so-agar medium was cultured in 10 mL LB broth overnight at 37°C. Cultured S. enteritidis in the LB broth was centrifuged at 4000 rpm for 5 min for bacterial precipitation. Then precipitated S. enteritidis was suspended in a micro centrifuge tube with a capacity of 1.5 mL with 300 μL of DNAse-RNAse-free distilled water by vortexing. Micro centrifuge tube was heated in boiling water for 15 min and then the suspension was centrifuged for 5 min at 10000 rpm. The supernatant was extracted carefully and made ready for electrophoresis. Plasmid DNA was isolated by an alkaline lysis procedure (Sinha et al., 1984).

PCR: The oligonucleotide primers used for the specific amplification of the SeßA gene, SeßA-F (5'-GGGCGATGCGTTACATCAGCA TGTGC-3') and SeßA-R (5'-GGTGTAGAATTCTTGTAGGATCTG GT-3') were designed by Riazi et al. (2003). A volume of 40 μL of the DNA template solution was added to 48 μL of reaction mixture containing ddH2O, 10 X PCR buffer, 50 mM MgCl2, 10 mM dNTPs, 50 pmole/L each primer, 5 U μL−1 Taq polymerase. Amplification was carried out in UK thermocycler (Model FTGRAD2D with temperature programme consisting of initial denaturation (3 min at 94°C), 30 amplification cycles (1 min at 94°C, 1 min at 52°C, 1 min at 72°C) and the final extension (10 min at 72°C). A volume of 5 μL of the PCR product was analyzed by electrophoresis in 1% agarose gel (Sambrook et al., 2001).

Restrictive endonuclease digestion: For confirming the PCR product, 2 μL of 10X buffer, 2 μL of DTT (1%), 2 μL of BSA (1%), and 0.25 μL of Avall (20 μL L−1) were added in a restrictive endonuclease system. The total volume was brought up to 20 μL with PCR products. Then it was incubated at 37°C for 1 h and checked in a 1% agarose gel electrophoresis at 4 V and developed with UV light. For confirming the SeßA-pTZ57R/T construct, the same method was used with PstI restriction enzyme.

Cloning of SeßA gene: The SeßA gene were obtained by PCR from chromosomal DNA isolated from S. enteritidis E3. The resulting PCR product inserted in the corresponding site of cloning vector, pTZ57R/T with Ferramentae Inst T/A clone™ PCR product Cloning Kit (# X1213).

Transformation: In order to insert recombinant vector (SeßA/pTZ57R/T) into the component cells (E. coli DH5α) Transform Aid™Bacterial Transformation System were used (Fermentase Inst T/A clone™ PCR product Cloning Kit). Transformed E. coli DH5α were grown at 37°C in LB-Ampicillin agar plates containing 50 μg mL−1 Ampicillin, 0.1 M IPTG (Isopropyl-I1-thiogalactopyranoside) and 20 mg mL−1 X-Gal (5-bromo-4-chloro-3-indoly-β-D-galactopyranoside).

Sequencing: Sequencing was performed by Korean Microgene Co. Ltd. with automation dideoxy chain determination method.

RESULTS AND DISCUSSION

Chromosomal DNA was extracted from S. enteritidis E3. The PCR reaction was performed and single band of 511 bp was amplified by SeßA-F/SeßA-R primers (Fig. 1). The product was checked with 1% agarose gel electrophoresis. The PCR product was performed by

![Fig. 1: Specific PCR for amplification of Salmonella enteritidis SeßA gene with primer pair SeßA-F/SeßA-R. L1 and L2 referred to control and Salmonella enteritidis E3 isolate PCR product, respectively. M = DNA marker (GeneRuler™ 1kb DNA Ladder #SM0313)](image_url)
Fig. 2a: Nucleotide alignment of *Salmonella enteritidis* E3 SefA gene with standard isolate (L11008). This alignment show that *Salmonella enteritidis* E3 carried out two nucleotide mutation in the positions of 89 and 387.

Fig. 2b: Translation alignment of *Salmonella enteritidis* E3 SefA protein with standard isolate (L11008). This alignment show that *Salmonella enteritidis* E3 carried out on amino acid mutation in the position of
Aval restriction enzyme and showed 217 and 281 bp fragments. The results indicated that SefA gene was amplified.

The PCR product was inserted in the corresponding site of cloning vector (pTZ57R/T) using Fermentase Ins T/A clone™ PCR product Cloning Kit.

In order to amplify the recombinant plasmids, E. coli DH5α bacteria was transformed with SefA/pTZ57R/T, via Transform Aid™ Bacterial Transformation system. Recombinant clones were identified by blue/white selection, since the vector is LacZ genetically marked. The correct insertion (SefA/pTZ57R/T product) was confirmed by restriction endonuclease analysis. The results showed that SefA/pTZ57R/T were correctly transformed in the E. coli DH5α.

Identification of the SefA/pTZ57R/T product was confirmed by sequencing. The sequence was compared with that published by the Genbank (L11008) which indicated that two point mutations exist in 89 and 387 nucleotide positions. Nucleotide and protein alignment with BLAST software showed that the sequence of the SefA gene derived from S. enteritidis E3, which was cloned in the pTZ57R/T vector, was 99% identical to that in the gene bank (L11008). The sequence of the SefA gene from S. enteritidis E3 differed only in two nucleotides (Fig. 2a) and this resulted in one amino acid difference (Fig. 2b). Thus, this method is simple and rapid and results obtained in less than 20 h proved to be highly specific and sensitive. The results of cloning and sequencing showed that the specimen from Kermanshah area was not different with other areas which are addressed in Genbank and these point mutation do not have a major effect on diagnostic of this bacteria.

REFERENCES


