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Short Communication

Difference and variation of the sef14 operon gene clusters in Salmonella pullorum


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SEF14 fimbriae are only found in some strains of serogroup-D Salmonella such as S. enteritidis, suggesting that SEF14 fimbriae may affect serovar-specific virulence traits. In this study, we found that prevalence of sefA, sefD and sefR genes in S. dublin and S. enteritidis was 100%. In 18 isolates of S. pullorum, the prevalence of sefA gene was 100%, while the prevalence of sefD and sefR genes was 38.9% (7/18), and 11 strains isolated after 1980s did not contain any gene sefD or sefR. Interestingly, among the 7 strains of S. pullorum before 1980s, the sefD sequence has a missing base pair at position 196 and caused open reading frame (ORF) shift, resulting in a stop codon (TAG) at position 71 amino acid residual (Leu of TTA at position 214–216 shift into stop codon of TAG at position 215–217). Unlike S. pullorum, all S. enteritidis and S. dublin tested could express SEF14 fimbriae in vitro.

Keywords: S. pullorum / Fimbriae / sef14 operon gene clusters / Variation

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Introduction

The existence of fimbrial structures on the surface of Salmonella enterica serovar, such as the plasmid-encoded fimbriae (PEF), long polar fimbriae (LPF), thin aggregative fimbriae (AGF) and type I fimbriae (FIM), has been recognized for many years [1–4]. Originally, Feu
trier et al. [5] detected a fimbriae from a human isolate of S. enteritidis, and biochemically characterized the fimbriae polypeptide subunit (Mr, 14,400), which was markedly lower than that reported for the type 1 fimbriae of S. typhimurium (Mr, 22,100) [5]. Ogunniyi et al. [6] reported that SEF14 fimbriae could illicit a strong, protective immune response, in the following study, some data shown that SEF14 fimbriae were not involved in primary attachment to murine or human-originated intestinal epithelia [7, 8]. Further results demonstrated that the SEF14 fimbriae were essential for full virulence of S. enteritidis in vivo, and that the SEF14 fimbriae mediated interactions between the bacteria and host phagocytes [9]. The role of SEF14 fimbriae in virulence remains to be further elucidated.

The sef14 fimbriae operon contains four structure genes (sefABCD) which required for the translocation and biogenesis of SEF14 fimbriae [9], both SEFA and SEFD are located on the cell surface and may play an important role in interaction between bacteria and host cells. sefA encodes the major subunit, sefD encodes the putative adhesin. And adjacent to sefD, there is an AraC-like regulatory protein encoded by sefR that activates transcription of the sef genes [10]. The gene sefA has been shown to have limited distribution among Salmonella serogroup-D and some species such as S. pullorum, S. gallinarum, and S. typhi possess the intact gene of sefA but fail to express SEF14 fimbriae [11]. In this study we focus on sefA, sefD and sefR of sef14 operon gene clusters of S. pullorum and S. enteritidis and investigate clues of different expression of SEF14 fimbriae between S. pullorum and S. enteritidis.

Materials and methods

Bacterial strains and culture

A total of 18 strains of S. pullorum, 11 strains of S. enteritidis and one strain of S. dublin were used in this study.
Standard procedures were used to prepare the bacteria. Colonisation factor antigen (CFA) broth (casomino acid 10 g/l, yeast extract 1.5 g/l, MgSO₄·7H₂O 0.05 g/l, MnCl₂ 0.005 g/l, pH 6.0) was prepared for SEF14 fimbriae expression [12].

S. pullorum isolates isolated in different times of years in the infected chickens were listed in Table 1, kindly provided by Yangzhou-based Poultry Institute, Chinese Academy of Agricultural Sciences, and of all 18 strains, 7 were isolated before 1980s. The S. enteritidis clinical isolates SE20, SE43, SE51, and SE57 from human gastroenteritis outbreaks were kindly provided by the Center for Disease Control of Yangzhou. The isolates MY, XJ were isolated from infected ducks (gifts from Dr. Cheng, College of Animal Science and Technology at Sichuan Agriculture University) and S. O9 isolate from the diseased chicken. The S. enteritidis standard strains 50336, 994, 3378 and 50041 were generous gifts from Dr. Schifferli, school of Veterinary Medicine at University of Pennsylvania and S. dublin (C79–84) was purchased from China Institute of Veterinary Drugs Control.

**PCR, DAN cloning and sequencing analysis**

The bacterial genomic DNA as PCR templates were extracted according to previous methods [13]. The presence of sefA, sefD and sefR gene of SEF14 fimbriae were detected by PCR amplification and each pair of oligonucleotide primers were used in the following: sefA F: CGC ATA TGG CTG GCT TGT TGG TAA C, sefA R: CGA CTA GTT TAG TTT TGA TAC TGC TGA A; sefD F: GAA TCA GTA TAA TTC GTC AAT ACC TAA G, sefD R: ATT CAA TTT CIG TCG CAT ATA TGC TTA T and sefR F: ATG TTG AAA AAA AAC GCC ATA A, sefR R: AGC ATA AAT GCA GCT TTT TCT C, respectively, which were designed according the reported sequence information [10, 14].

PCR products were subjected to horizontal gel electrophoresis in 1.5% (w/v) agarose, and the size of PCR products were determined based on DL2000 marker (Takara biotechnology). All PCR products with expected size of molecular weight were purified and cloned into pMD18-T easy vector (Takara biotechnology) for sequencing by commercial service (Takara biotechnology and service) and analysis with the help of biosoftware DNASTAR (Dnastar).

**Isolation and identification of SEF14 fimbriae**

The gene sefA was cloned into plasmid pET22b+ (Novagen) and constructed recombinant plasmid, pETsefA. After pETsefA was transformed to host cells E. coli BL21 (DE3) and 0.1 mM IPTG induce for optimal condition, the recombinant BL21 (DE3) with pETsefA expressed the soluble fusion protein of SEFA protein and polyhistidine-tag (rSEFA-his) with approximate size of 15.2 kDa which were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western-blotting analysis with mouse anti-polyhistidine monoclonal antibody (Invitrogen). The fusion protein was purified by Protino Ni-TED 2000 packed columns according the purification manual (Macherey-Nagel). The mouse sera with high titer of anti-SEFA were made after being immunized with the purified rSEFA-his protein.

SEF14 fimbriae were extracted according the report-ed methods with some modification [15]. Briefly, bacteria were grown statically in 200 ml of CFA medium at 37 °C for 50 h, harvested by centrifugation, and pellets were suspended in 10 ml of 0.15 Methanolamine buffer (pH 10.5). Fimbriae were separated from the cells at room temperature by shearing bacteria in a blender for three minutes, after which cells and cellular debris were removed by centrifugation (12,000 × g, 15 min, and 4 °C). The supernatant was dialyzed overnight against 10 mM Tris-Cl (pH 7.5) containing 0.2% SDS to precipitate fimbriae SEF14. These proteins were resolved by 12% SDS-PAGE, Western-blotting was conducted as described and the mouse anti-rSEFA serum was used as the first antibody, horse-radish peroxidase (HRP)-conjugated goat anti-mouse IgG (Sigma) for the secondary antibody [16].
Table 1. Prevalence of sefA, sefD and sefR genes in S. pullorum.

<table>
<thead>
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<td>+</td>
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+, positive; –, negative; year, when the strain was isolated

Result and discussion

Based on molecular weight and DNA sequence the prevalence of sefA, sefD and sefR genes in S. dublin and S. enteritidis was 100%. In S. pullorum sefA gene was detected in all 18 isolates of S. pullorum, while sefD and sefR genes were only detected in 7 S. pullorum strains those isolated before 1980s. The prevalence of sefD and sefR was 7/18 (38.9%) on S. pullorum strains (Table1). The sequence of sefA, sefD and sefR genes in S. enteritidis were identical with those sequences in NCBI GenBank data which accession number were L11008, U07129 and AF233854, respectively. The sequence of sefA gene from 18 isolates of S. pullorum was completely identical to sefA gene from S. enteritidis. Most interestingly, 7 isolates of S. pullorum containing sefD gene has a missing base pair at position 196 which caused ORF shift, resulting in a stop codon (TAG) at position 71 amino acid residual (Leu for TTA at position 214–216 shift into stop codon for TAG at position 215–217) (Fig. 1).

Edwards et al. [9] suggested SEF14 fimbrial subunits from the periplasm of bacteria were exported across the cytoplasmic membrane in a secretory-dependent manner. Using a genetic approach with well defined, isogenic mutants of ΔsefA strain and ΔsefD strain, expression of SEF14 fimbriae in different mutants showed that export of SEFD occurred in the absence of sefA gene (ΔsefA strain) and export of SEFA did not occur in the absence of sefD gene (ΔsefD strain) [9]. They proposed that sefD encoded the adhesin subunit of SEF14 fimbriae and the translocation of sefD is a prerequisite for

![Figure 2 A. SDS-PAGE analysis for SEF14 fimbriae from different salmonella strains B. Western-blotting analysis for SEF14 fimbriae and anti-rSEFA serum as the first antibody. M. Molecular weight of protein marker (117, 85, 48, 34, 26, 19 KD); lane 1. S. enteritidis; lane 2. S. pullorum; lane 3. S. Dublin.](image-url)
the export of sefA across the outer membrane [9]. In this report, although 18 isolates of S. pullorum contain the intact sefA gene, but no SEF14 protein expression was detected. The missing base pair at position 196 in sefD gene, or absence of sefD gene in all 18 isolates may be the reason that they did not express SEF14 fimbriae. In the following study, through SEF14 fimbriation culture, fimbriae extraction, isolation and identification, the fimbriae expression in different Salmonella strains was tested. Based on SDS-PAGE analysis, there were 14.3 KD protein bands of major subunit SEFA in lane1 and lane3 for all strains from S. enteritidis and S. dublin, respectively, but not in lane2 for S. pullorum (Fig. 2A). And the result of Western-blotting further confirmed that the kind of protein extracted from S. enteritidis and S. dublin could be recognized by anti-rSEFA serum, there was no band showed in lane2 for S. pullorum (Fig. 2B). This illustrated that we extracted SEF14 fimbriae from S. enteritidis and S. dublin successfully, and S. enteritidis and S. dublin could express SEF14 fimbriae in the surface of bacteria, but not S. pullorum. This result was identical with the prevalence of sef genes and their sequencing in S. pullorum.

Very interestingly, in this study we found that the isolated S. pullorum strains before 1980s all contain defective sefD and sefR genes, and those strains isolated after 1980s just contained sefA gene, but no sefD and sefR gene. Based on the data of the difference and variation of sef14 operon gene clusters between S. enteritidis and S. pullorum, we may explain why SEF14 fimbriae in S. pullorum could not be expressed.

By now we are not sure the role of SEF14 fimbriae as a virulence factor and the significance of the SEF14 antigen existed in some strains of Salmonella. And this kind of mutant isolates or mutants of sef gene unable to express SEF14 fimbriae may help to answer some of these questions.

Acknowledgements

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References


