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Review

The role of SEF14 fimbriae in pathogenesis and enhancing the immunity of *Salmonella enteritidis*

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Food safety is an important component of effective prevention and control strategy for *Salmonella enteritidis* infection. The control strategies have been implemented in poultry with ultimate aim to reduce S. *enteritidis* out breaks in human. SEF14 fimbriae are expressed on strains of serogroup D *salmonella* and contribute their survival in macrophages. SEF14 fimbriae are immunogenic in the *S. enteritidis* infected chicken. SEF14 antibodies is protective in mice, IgA and IgG response were found in sera of immunized chicken with SEF14 antigen. In this review we concluded that the immunogenecity of SEF14 antigens in the infected birds may serve as component of an effective sub-cellular vaccine for poultry.

Key words: *Salmonella entertidis,* SEF14 fimbriae, pathogenicity.

INTRODUCTION

The *Salmonella serovars Enteriditis* and *Typhimurium* are the most important causes of food-borne diseases due to the infections occurring following the direct or indirect consumption of contaminated food products (Hedberg et al., 1993) The prevalence of *S. enteritidis* has dramatically increased worldwide, it has been reported as the most common serotype in the United States and about 1,000 people die of the disease each year (Rabsch et al*.,* 2007). Foods of animal origin, such as meat, dairy products and eggs, have been implicated in outbreaks of human salmonellosis. The presence of *Salmonella* in animal products presents a risk when these products are undercooked, mishandled, or allowed to cross contaminate other foods during food production or preparation (El-Gazzar et al., 1975). The success of *S. enteritidis* was largely due to their ability of adherence and invasion into several kinds of cells. Vertical transmission of *S. enteritidis* has been demonstrated (Gast et al*.,* 1990). Fimbriae are used for colonization and invasion into host cells, and have drawn considerable interest because fimbriae can serve as potential immunogens against many pathogenic bacteria that colonize epithelial surfaces (Rajashekara et al., 2000). SEF14 fimbriae are only found in *S. enteritidis* and closely related serovars, suggesting that SEF14 fimbriae may affect serovar-specific virulence traits (Edward et al., 2000).

For example, SEF14 fimbriae may contribute to the adherence of *S. enteritidis* to mouse epithelial cells and passive administration of SEF14 antibodies is protective in mice (Thorns et al., 1996). On the other hand, SEF14 fimbriae were contributed that *S. enteritidis* survive in kinds of host cells such as macrophages (Edwards et al., 2000). In this review, we focused on the SEF14 fimbriae and its role in pathogenesis and enhancing the immunity of *S. enteritidis*.

CLASSIFICATION OF *SALMONELLA* FIMBRIAE

The existence of fimbrial structures on the surface of *Salmonella* has been recognized for many years. They are characterized into four main types based on their morphology and ability to mediate erythrocyte agglutination. Type 1 fimbriae are rigid surface organelles with a diameter of 7 nm which mediates agglutination of erythrocytes in the absence of a-D-mannose (Old, 1972). They are ubiquitous among *Salmonella* species; however

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the published evidence suggested that they are antigenically distinct types (Clegg and Gerlach, 1987). Type 2 fimbriae are morphologically similar to type 1 but lack the ability to agglutinate erythrocytes (Duguid et al., 1966). They have been found only on a few serotypes and may represent a mutant form of type 1 fimbriae (Clegg and Gerlach, 1987). Type 3 fimbriae are smaller, more flexible structures with a diameter ranged between 3 to 5 nm and are able to mediate the agglutination of tanned animal erythrocytes in the presence of a-D-mannose (Duguid et al., 1966). It described on a few serotypes of *Salmonella*: *S. enteritidis*, *S. typhimurium* and represent a number of antigenically distinct structures. Type 4 fimbriae are morphologically similar to type 3 and are able to mediate the agglutination of fresh erythrocytes in the presence of a-D-mannose, it is described on *S. typhimurium*.

S. enteritidis expresses an array of fimbrial antigens on its surface including SEF14, SEF17, SEF18 and SEF21. They also have genes that express bundle-forming pilus (BFP), long polar fimbriae (LPF) and plasmid-encoded fimbriae (PEF) (Thorns et al., 1996). Although *Salmonella* serovars share many fimbrial operons, a few fimbriae are limited to specific *Salmonella* serovars. SEF14 fimbriae are restricted to group D *Salmonella* and the genes encoding this virulence factors are acquired relatively (Edwards et al., 2001). Some fimbriae lack the ability to agglutinate erythrocytes, these include SEF14, SEF17 that cannot be easily ascribed to any of the existing fimbrial types.

DETECTION OF SEF14

SEF14 was first isolated from a human isolate of *S. enteritidis*, the polypeptide subunit (Mr, 14,400), was lower than that reported for the type 1 fimbriae of *S. typhimurium* (Mr, 22,100) (Feutrier et al., 1986). These fimbriae are less than 5 nm in diameter and they do not have hemagglutinating activity. Evidence shows that the fimbriae carry an epitope present on all *S. enteritidis* strains and certain strains of *S. dublin* and *S. moscow*, but not on *S. typhimurium* strains and other group D *Salmonella*. It differs from all previously described structures of *Salmonella* (Thorns et al., 1990). Under certain growth conditions, *S. enteritidis* produced both SEF14 and SEF21. A simple method for the purification of both fimbriae was developed by using the different biochemical properties of these fimbriae (Müller et al., 1991).

GENETIC CONFIGURATION OF SEF14

Fimbrial proteins are secreted into the periplasm by means of the general secretory system; two accessory proteins are assisting the construction of fimbriae. First, the fimbrial subunits are bound by a chaperone in the periplasm to prevent premature aggregation and then the sub-units are translocated across the outer membrane by an usher protein. In all fimbrial systems that have been studied, the genes encoding the chaperone and usher are located in the same operon as the major subunit. It is expected that *S. enteritidis* contains a similar number of fimbrial operon to these other enteric bacteria.

The *sef* operon contains four structural genes (*sefABCD*) required for the translocation and biogenesis of SEF14 fimbriae: *sef*A encodes the major subunit, *sefB* and *sefC* encode the chaperone and usher, respectively, and *sefD* encodes the putative adhesin. Adjacent to *sefD*, there is an AraC-like regulatory protein (encoded by *sefR*) that activates transcription of the *sef* genes (Collighan and Woodward, 2001).

The gene *sef*A, encoding the major subunit of SEF14 has been shown to have limited distribution among *Salmonella* serotypes belonging to serogroup-D. SEF14 fimbriae are present in *S. enteritidis, S. dublin, S. moscow,* and *S. blegdam* (Thorn et al., 1992). Though many of the serogroup-D *Salmonella* such as *S. typhi, S. gallinarum,* and *S. pullorum* possess the intact gene, but they fail to express SEF14 fimbriae (Turcotte and Woodward, 1993). The *sefABC* genes are transcribed as part of a single mRNA transcript. *sefB* and *sefC* were not expressed in the absence of *sefA*, and no transcription start sites or promoters were found immediately upstream of *sefB* or *sefC*. Furthermore, the 5' ends of several mRNA transcripts were mapped to the region upstream of *sefA*. Therefore, transcription of *sefB* and *sefC* is initiated from the *sefA* promoter region. The significance of multiple minor transcription start sites upstream of *sefA* is not clear (Clouthier et al., 1993). *Sef* operon is transcribed as a single mRNA transcript in presence, a posttranscriptional mechanism must exist which regulates the relative production of the fimbriae protein, the chaperone protein, and the outer membrane protein. One potential mechanism involved is RNase E-dependent endonucleolytic cleavage of the primary mRNA transcript at adenine-uracil-rich regions (Nilsson and Uhlin, 1991).

EXPRESSION OF SEF14 FIMBRIAE

Expression of the *sef* genes was optimal during growth in late exponential phase and repressed during the stationnary phase. The regulation is coordinated by the RpoS sigma factor (Edward et al., 2001). The environmental signals that regulate SEF14 fimbrial elaboration include temperature, surface contact, oxygen tension, osmolarity and ionic environment (Walker et al., 1999). ELISA specific for the detection of SEF14 fimbriae was used to assess the effect of temperature and pH upon their elaboration by isolates of *S. enteritidis* in planktonic growth and on the surface of two-dimensional gradient agar plates. It reported that SEF14 fimbriae were elaborated in planktonic growth at 37°C and at pH 4.77; whereas on agar gradient plates SEF14 fimbriae were elaborated poorly

(Walker et al., 1999). But in our lab, we showed that the best condition for SEF14 expression is 37℃ within CFA broth steadily culturing for 50-72 h (Unpublished data).

ROLE OF SEF14 FIMBRIAE AND PATHOGENICITY

Most fimbriae such as the common type 1 fimbriae play a critical role in virulence by allowing bacteria to interact with host cell and other solid substrates, but there are some fimbriae whose distribution is limited may provide specific functions required in virulence. Edwards et al. (2000) constructed *sefA* and *sefD* mutant to explore the role of SEF14 fimbriae. In his report, we found that the minor subunit, *sefD* is essential for SEF14 fimbriae function *in vitro*. He proposed that *sefD* encodes the adhesin subunit of SEF14 fimbriae. The translocation of *sefD* is a prerequisite for the export of *sefA* across the outer membrane; thus, *sefD* is probably located at the tip of the fimbrial shaft.

The major SEF14 subunit *sefA* is not required for the virulence of *S. enteritidis*, indicating that the tip of the fimbrial structure composed of *sefD* subunits is probably sufficient for successful interactions with phagocytes (Edwards et al., 2000). It is possible that either the major subunit is redundant or *sefD* is presented on another fimbrial shaft, or that *sefD* tip structures can successfully fulfill all the functions required of the SEF14 fimbriae in the absence of *sefA*. When there is insufficient *sefA*, *sefD* may form a fibrillar structure on the bacterial cell surface similar to that described for other fimbriae (Jones et al., 1995).

To date, the role of SEF14 fimbriae in virulence has been controversial. It has been showed that SEF14 fimbriae are not involved in primary attachment to intestinal epithelia, but SEF14 fimbriae can illicit a strong, protective, immune response (Edward et al., 2000). Liposome-associated fimbriae antigens (SEF14 and SEF21) were prepared for intraocular immunization to seek protective efficacy for intestinal infection with *S. enteritidis*. Evidence showed that an IgA and IgG responses were found in the intestinal tract and in sera of treated chickens. And antibody-secreting lymphocytes were detected in the Harderian gland of immunized chickens as determined by enzyme-linked immunospot assay (Li et al., 2004).

Intraocular immunization with liposome-associated SEF14 and SEF21 elicits both systemic and mucosal antibody responses and *S. enteritidis* colonization in the intestinal tract and excretion of *S. enteritidis* in the feces are suppressed by immunization (Li et al., 2004). In order to evaluate its use in the specific detection of chicken flocks infected with *S. enteritidis*, the SEF14-DAS ELISA (A double-antibody sandwich enzyme-linked immunosorbent assay) was described. It successfully discriminated between chickens experimentally infected with *S. enteritidis* and those infected with *S. panama* or *S. typhimurium*, although the SEF 14 responses in adult birds infected with *S. enteritidis* were detectable but low. In contrast, ELISA used to detect antibodies to lipopolysaccharide (LPS) and flagella were unable to discriminate between the infected groups of chickens and adult birds infected with different *Salmonella* serotypes (Thorns et al., 1996).

PREVENTIVE MEASURES

Many control strategies have been implemented to reduce the prevalence of *S. enteritidis* infection in poultry with the ultimate aim of reducing *S. enteritidis* outbreaks in humans (Rajashekara et al., 1998). This included the practice of adequate rearing programs to control the vertical transmission in chicken breeder flock. Fimbriae have been shown to be involved in colonization and in adherence to specific host target tissues in the early stages of infection (Bäumler et al., 1996) and fimbriae also can serve as potential immunogens against many pathogenic bacteria that colonize epithelial surfaces (Krogfelt et al., 1991). Flagellin, porins, OmpA, SEF14 and SEF21 fim-briae are immunogenic in the *S. enteritidis* infected hens. The immunogenicity of these antigens in infected birds provides promise that they may serve as components of an effective sub-cellular vaccine for poultry Salmonellosis (Javier et al., 2004). Some recombinant strains were constructed to evaluate the possibility of protection against *S. enteritidis* infection in chickens. For example, the *sefA* gene was cloned into a temperature-sensitive expression vector and then transformed into a non-pathogenic, avirulent strain of *Escherichia coli*. The recombinant strain was used as a vaccine to elicit specific immune response against the *sefA* protein of *S. enteritidis* in 1-day-old chickens. In addition, IgA against the *sefA* protein was detected in intestinal secretions from treated birds at 7 days post-treatment and in bile samples from 14 to 21 days post-treatment by ELISA. Non-treated birds did not show any evidence of intestinal colonization by the recombinant strain or anti-SEFA IgA response in their bile or intestinal secretions (Lopes et al., 2006). Rajashekara et al. (2000) used *S. enteritidis* fimbrial antigens as vaccine candidates to reduce the colonization and prevalence of the bacterium in poultry.

Conclusion

The research on SEF14 fimbriae protein has developed with the progress of molecular biology techniques. How-ever, many outstanding questions concern with pathogennic mechanism and specific immune protection mechanism were in debts. Therefore, according to the limited distribution of SEF14 fimbriae on group D *salmonella*, we can establish new diagnostic techniques and new method to distinguish different Salmonella serogroup. In addition, we can develop genetic engineering vaccine for future use.

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