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Roles of Fimbriae in the Bacterial Pathogenesis

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Fimbriae are hairlike appendages ranging from 2 to 8 nm in diameter on the surface of bacteria. *E. coli* and *Salmonella* spp. express a wide variety of different fimbriae exhibiting different binding specificities. A single bacterial isolate can almost express multiple fimbrial types. Fimbriae containing 100 to 1,000 per cell are thinner and generally shorter and more numerous than flagella. The bacterial cell containing fimbriae can adhere with inanimate surfaces and eukaryotic or prokaryotic cells. Tight contact, called adherence, precedes to colonization of surfaces and invasion of eukaryotic cells by the bacteria. Fimbriae are relatively well studied in *E. coli* and *S. enterica.*

In the analyses entire nucleotide sequences of *Salmonella* genome, 13 putative fimbrial operons, such as *agf (csg), fim, pef, lpf, bcf, saf, stb, stc, std, stf, sth, sti* and *stj*, were observed in *S. typhimurium* genome. Evidences for *in vitro* expression of these operons are currently only available for *agf,* and. The remaining 10 fimbrial operons of *S. typhimurium* appear to be poorly expressed when bacteria are grown under standard laboratory conditions, and no information is available about the binding specificity of the encoded adhesions.

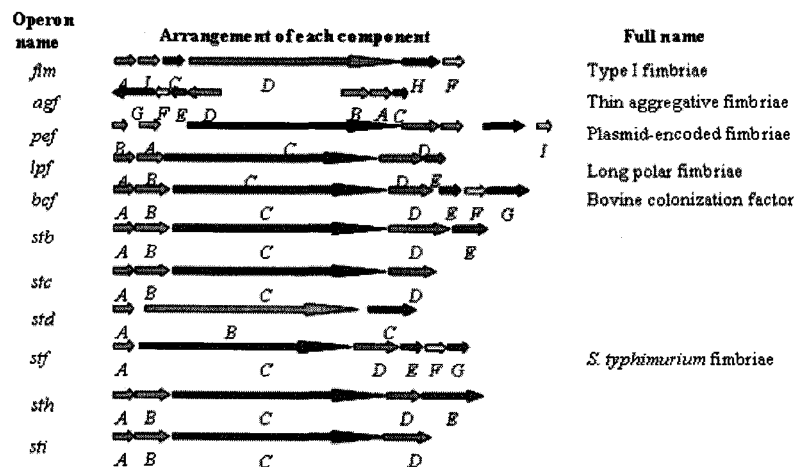

Fig. 1. Putative fimbrial operons in *S. typhimurium* genome.

The primary amino acid sequences of a number of fimbrial major subunit proteins have been reported to provide a basis for the classification scheme. Major subunits of class 1 fimbriae including Pap and type 1 have two conserved cysteine residues spaced by 38 to 43 amino acids from each other and may form a cystine bridge. Class 2 fimbriae include F1845 as well as adhesions that do not form fimbriae, the afimbrial adhesions AFA-I, AFA-III, and Dr (O75X). Class 3 fimbriae, which include K88, CS31A and F41, are the lack of conserved Cys residues and thus distinct from fimbrial classes 1 and 2. The bundle-forming fimbrin BfpA, the sole member of class 4, shares the highest amino acid sequence identity with the toxin-coregulated pilus (TCP) from *Vibrio cholerae*. The CFA/I and CS1 fimbriae share 55% sequence identity and are distinct from the other fimbrial classes. These fimbriae lack cysteines and COOH-terminal Tyr residues and are designated as class 5.

Nucleotide sequence analyses of *S. typhimurium* and *S. typhi* chromosomal sequences between the ferrichrome operon and *hemL* had identified a novel *S. typhimurium* fimbrial operon called *stf* operon, which exhibits a high level of similarity to sequences encoding *Proteus mirabilis* mannose-resistant. The fimbrial operon was absent from the *S. typhi* genome. The genetic organization of *stf* operon, *stfACDEFG*, strongly resembles those of other enteric fimbrial operons. The StfA is a major structural subunit for Stf fimbriae. The StfC and stfD are outer membrane fimbrial ushers and chaperones, respectively. The others encode predicted proteins with similarities to minor fimbrial subunits. Over 30 different operons encoding virulence-associated surface structures of gram-negative bacteria have now been identified as members of a family using the chaperone-usher protein-assisted assembly pathway. The prototype of this pathway has been the PapD chaperone-PapC usher-mediated assembly of Pap pili in *E. coli*.

The Stf fimbriae was grouped into the class I when compared with primary amino acid residues of major subunits, StfA. In order to investigate condition of *stf* operon expression, we have constructed a strain containing a transcriptional fusion of *lacZYA* reporter gene to *stfA* gene on chromosome, resulting in χ8532. The strain χ8532 appeared little or no expression of *lacZ* reporter gene under normal culture conditions on MacConkey agar plates. However, with long time incubation of χ8532, we obtained 21 isolates exhibiting β-galactosidase activity. Those were appeared approximately 1 colony frequency during 34 generations. To characterize promoter regions of the isolates, upstream regions of *stf* operon were amplified by PCR with various primer sets. Followings are the results of the PCR; (1) Partial deletion in upstream regions of the operon may occur in 2, 4, 6, 10, 12, 13, 14, 15, 16, 18 and 21; (2) No changes of the DNA in length may occur in 17 and 20; (3) Remains contain DNA region which couldn't amplify PCR. To investigate deleted or mutated regions, we performed nucleotide sequencing of cloned DNA fragment from isolates 2, 4, 6, 10, 12, 13, 14, 15, 16, 17, 18, 20 and 21. Results of nucleotide sequence analyses didn't exhibit any regular conformation on deleted or mutated regions such

as inverted repeat sequences or any other specific sites. Among them, isolate 19 showed the highest β-galactosidase activity, while isolate 17 the lowest. Analyses of transcripts through RT-PCR with RNA samples isolated from 15, 17, and 21 were directly proportional to β-galactosidase activity. Although we detected Stf fimbriae on cell surface with dot blotting, we couldn't find normal fimbrial structure by TEM or immuno-gold labeled TEM.

A strain defecting expression of StfA showed attenuation in the virulence examination. To confirm the roles of Stf fimbriae in the pathogenesis, repeat animal experiments are in the middle of ongoing.

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