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Salmonella Fimbriae: What is the Clue to Their Hairdo?

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Abstract

Fimbriae are important virulence factors for *Salmonella* pathogenesis. They mediate adhesion to host cells (including plants), food, stainless steel and much more. The fimbrial systems are organised in gene clusters of four to fifteen genes that code for structural, assembly and regulatory proteins. There are three kinds of fimbriae depending on their mode of assembly. The chaperone/usher (CU) fimbriae use a dedicated chaperone and usher protein to coordinate the subunit biogenesis on the cell surface. The curli fimbriae are assembled by nucleation/precipitation pathway. The type IV fimbria assembly requires a transmembrane apparatus and ATP to energise the reaction. Several fimbriae are conserved among *Salmonella* serovars, while some are present in a limited set or only specific serovars. Expression and regulation of fimbrial genes are not well understood, and most *Salmonella* fimbriae are poorly expressed during in vitro culture, which further complicates research concerning their regulation and role during infection. However, *Salmonella fim* gene cluster, coding for type-1 fimbriae, was widely studied and presents its own set of regulators. Investigating fimbrial distribution, expression and regulation will further elucidate their roles in bacterial pathogenesis and host specificity. Furthermore, fimbriae are important for developing efficient diagnostic tests and antimicrobial strategies against *Salmonella*.

Keywords: fimbriae, adhesion, chaperone/usher, curli, type IV fimbria, fimbriome, *fim*

1. Introduction

Multiple virulence factors are implicated in *Salmonella* pathogenesis. These factors include type 3 secretion systems (T3SS) encoded in *Salmonella* Pathogenicity Islands (SPI)-1 and SPI-2, other SPIs, flagella, capsule, plasmids and adhesion systems [1, 2]. Among those factors, fimbriae represent a major player in pathogenesis and a source of diversity for *Salmonella*

serovars. Fimbriae are the most common adhesion systems and are differentially expressed and found in a specific pattern among each serovar [3, 4].

Historically, the first observation of fimbriae was described in 1901 in *Bacillus anthracis* by Hinterberger and Reitman which hypothesised that the filaments were implicated in nutrients acquisition [5]. Then, in 1949, Anderson suggested that the filaments were artefacts due to sample preparation for electron microscopy [6]. However, many other studies contradicted Anderson and confirmed the presence of non-flagellar appendages on the bacterial surface. In 1950, Houwink and Van Iterson observed the appendages and described them as shorter and more rigid filaments than the flagella from *Escherichia coli* and suggested that the fibres were implicated in attachment to surface [7]. The name fimbria (Latin word for fibres) was suggested in 1955 by Duguid et al. to describe the filamentous structures [7, 8]. The term fimbria is preferable to use to describe non-flagellar filaments than pili, which is used to designate structures implicated in conjugation [9, 10]. In 1966, Duguid et al. classified fimbriae in seven types (types 1–6 and F) according to the morphology and haemagglutination patterns. However, another classification, based on serology, better predicted genetic relatedness of fimbrial antigens. Nowadays, fimbriae are designated by the mode of assembly of the fibril [8].

A specific fimbrial gene cluster (FGC) encodes for the structural, assembly and sometime regulatory proteins required for the production of the filamentous adhesive appendage on the bacterial surface. FGCs are usually composed of four to fifteen genes [10, 11]. An average of 12 FGCs by strains was observed in *S. enterica*. Despite that all *Salmonella* genome harbours multiple FGCs, very few are characterised so far. Most fimbriae are poorly expressed under laboratory conditions, and the functional redundancy complicates their studies [10]. However, fimbriae are implicated during infection and in a variety of other roles, like biofilm formation, seroconversion, haemagglutination, cellular invasion and macrophage interactions [2, 7, 12–16]. In mice model, *S.* Typhimurium fimbriae demonstrate a role in intestinal cells attachment, caecum colonisation and persistence in gut [17–19]. Moreover, fimbriae are important determinants of host adaptation by *Salmonella* [20].

In this chapter, an overview of *Salmonella* fimbriae is presented. First, the three pathways for fimbrial biogenesis (CU, precipitation/nucleation, type IV fimbriae) are described. Second, the distribution of fimbrial genes among *Salmonella* subspecies and serovars is presented. Third, the regulation of fimbrial genes is described and *fim* FGC regulation is detailed. Finally, the use of fimbriae as diagnostic and therapeutic tools is discussed.

2. Fimbrial biogenesis pathways

Three pathways for fimbrial assembly exist in *Salmonella*, the chaperone/usher (CU), the nucleation/precipitation and the type IV pathway [21]. Fimbriae of the CU pathway employ dedicated chaperones and ushers for the fimbrial assembly. The nucleation/precipitation pathway forms an aggregative fibre by precipitation of the subunits in the presence of the nucleator in the extracellular environment. Finally, the type IV fimbrial pathway uses complex machinery

for the fimbriae formation and needs ATP to drive the reaction of assembly. Furthermore, the type IV fimbriae can retract and reverse its assembly [21].

The three pathways produce quite different fimbriae. CU fimbriae have the classic fimbrial shape with the repetition of major subunits emerging from the usher inserted in the outer membrane. The major subunits can be accompanied by minor subunits and/or adhesins [8]. The fimbriae produced by the nucleation/precipitation pathway have an aggregated shape, due to the precipitation of major subunits together. This kind of fimbriae is highly stable and hardly depolymerised [22]. The type IV fimbriae anchor in the inner membrane and are prolonged by the repetition of the major subunit (pilin) through the periplasm and the outer membrane reaching the extracellular medium [23]. Here, the three fimbrial assembly mechanisms will be detailed.

2.1. Chaperone/usher pathway

The CU fimbriae represent the largest and most diversified class of adhesion systems [24, 25]. Multiple CU fimbriae are present in *Salmonella* suggesting a functional redundancy [23, 26]. The assembly is characterised by an interaction between the subunits, a periplasmic chaperone and an outer membrane usher in order to form a mature fibre (**Figure 1**) [27]. Each fimbria produced by this pathway has its own unique and specific chaperone and usher [11]. Usher sequence is a good discrimination tool and is used to subdivide the CU fimbriae into six phylogenetic clades (α, κ, π, σ, γ, β) [10, 26].
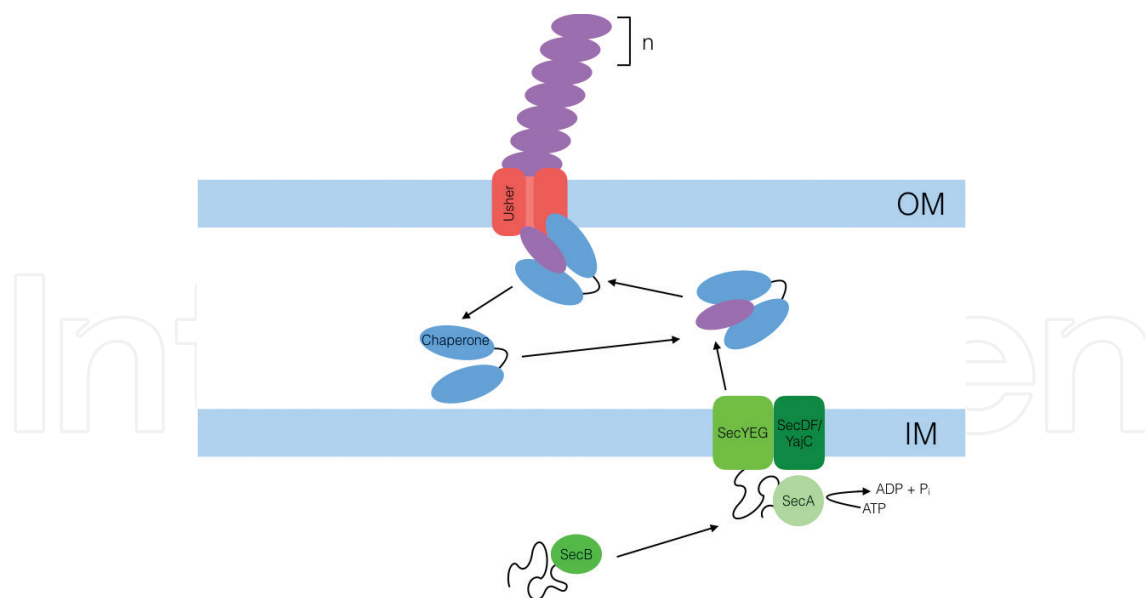
Figure 1. Chaperone/usher pathway. The subunit proteins are synthesised in the cytoplasm and translocated through the periplasm via SecYEG implying also SecDF/YajC inner membrane proteins. When the signal peptide is cleaved from the subunit, the chaperone protein complements the missing strand of the subunit in a process called donor strand complementation. The energy from the folding of the subunit is preserved by the chaperone. The chaperone drives the subunit to the usher and exchanges the donor strand. The subunit is then translocated by the usher to the extracellular medium and added to other subunits to form the fibril. IM = inner membrane; OM = outer membrane.

The biogenesis of the CU fimbriae begins with the production of the subunits in the cytoplasm and their export through the inner membrane by the general secretory pathway (GSP) [23, 27, 28]. It consists in a post-translational translocation implying the SecYEG complex and SecDF/YajC proteins. When the pre-protein is produced, it can be targeted directly to the accessory factor SecA or transported to SecA by the general chaperone SecB. Then, SecA catalyses the hydrolysis of ATP to energise the translocation through SecYEG. Use of ATP, in combination with proton-motive force, triggers the transport of the pre-protein to the periplasm. During the translocation across the inner membrane, the N-terminal signal peptide is cleaved by periplasmic peptidases [27, 29]. To prevent early folding of the subunits, the fimbrial chaperone instantly forms a complex with the translocated subunit in the periplasm [30].

Fimbrial chaperone shares conserved structural features with the general periplasmic chaperones [30]. They are formed of two β-sheet domains oriented to produce an L-shaped molecule and together form a β-barrel. Each domain has an immunoglobulin-like fold and is composed of seven primary β-strands [30–32]. Hydrophobic residues are alternated in the seven strands, facing the internal part of the barrel. These residues form the hydrophobic core of the domain that is implicated in the binding of the subunit. The fimbrial chaperones have an extended loop that lies at the extremity of one arm of the L-shaped molecule. This loop contains a conserved motif that is involved in the complex formation between the chaperone and subunits [30]. The subunit and the chaperone have a similar structure, but the subunit is missing the seventh β-strand of the C-terminal extremity [28]. The chaperone transfers the missing β-strand to the subunit to complete its structure: this mechanism is called the donor strand complementation [25]. The chaperone preserves the folding energy of the subunit to drive the last steps of the assembly due to lack of energy source (ATP) in the periplasmic space [33]. The chaperone also prevents premature fimbrial formation in the periplasm and primes the assembly through the usher [30, 34].

Then, the uncapping of the chaperone by the usher exposes the interactive surface of the subunit to the outer membrane usher and assembly of subunits at the surface can occur [33]. The transfer of the subunit from the chaperone to the usher happens very rapidly in vivo. In the absence of the usher in vitro, only a slow and inefficient assembly was observed. This suggests that the uncapping of the chaperone is important for the efficiency of mature fimbriae assembly [28, 30]. An interaction between the usher and the subunit and also between the usher and the chaperone is required [31]. This triangular interaction is important for the usher to discriminate subunit-loaded from unloaded chaperone [33]. Fimbrial usher forms a ring in the outer membrane with a transient twin-pore of 2–3 nm diameter to allow passage of subunits to the extracellular environment [35]. The usher catalyses fimbrial polymerisation by involving donor strand exchange where the N-terminal sequence of the subunit is replaced by a short sequence of the last subunit in the polymerised fibril with a zip-in-zip-out mechanism [33]. This step is triggered in part by the chaperone required for the strand exchange between the new subunit and the forming fimbria. The quaternary structure of the subunit is achieved when the protein passes through the pore. The final morphology and structure (rigid or flexible), the length (1–3 μm) and width (2–10 nm)

of the fibre of the CU fimbriae depend on the subunits composition and the interactions between subunits [10, 33].

2.2. Nucleation/precipitation pathway

Curli fimbriae were initially discovered in *Escherichia coli* and are very conserved among the *Enterobacteriaceae* family, compared to any other types of FGC. The amyloid fibrils are particularly known for their role in biofilm formation and its recognition by the immune system [36]. The FGC for curli is named *csg* (curli subunit gene) for *E. coli* and *agf* (thin aggregative fimbriae) for *Salmonella*, but the term *csg* is now commonly used for *Salmonella*. Curli formation depends on two divergent operons, *csgBAC* and *csgDEFG*. The *csgBAC* genes encode for CsgA, the major subunit, CsgB, the nucleator, and CsgC, an oxidoreductase of unknown function. The *csgDEFG* genes encode for the transcription regulator of the operon (CsgD) and for the assembly proteins located in the periplasm (CsgE) or in the outer membrane (CsgG and CsgF) [37].

The curli assembly mechanism is characterised by the exportation of the subunits and their precipitation to each other in the presence of a nucleator that fixes the fibril on the bacterial surface. Exportation of curli proteins also uses the GSP to pass through the inner membrane to the periplasm. Then, the CsgA and CsgB proteins are secreted by the lipoprotein CsgG. CsgG is composed of nine anticodon-binding domain-like units that form a 36-stranded β-barrel complex that is inserted in the outer membrane. CsgG forms a pore in the outer membrane that permits the passage of the subunits and the nucleator. CsgG is accompanied by the accessory proteins CsgE and CsgF. CsgE is a specificity factor that forms a nonameric adaptor that binds to CsgG and closes the periplasmic space. The presence of CsgE optimises the uptake of CsgA by CsgG and translocation of CsgA [38]. CsgF helps the nucleation activity of CsgB. It was suggested that CsgF has a role in specific localisation and/or chaperoning of the nucleator, so CsgB will reach its full activity. Moreover, CsgF depends on CsgG and CsgE for its stability [39].

Once at the bacterial surface, the nucleator polymerises the subunits together into thin aggregative fimbriae (fibrils). This process happens only in the extracellular environment and requires the presence of the nucleator CsgB to polymerise CsgA into a filament. CsgA proteins fold into an insoluble cross β-sheet molecules [26]. CsgB anchors the curli fimbriae on the surface of the bacterial cell (**Figure 2**). In *E. coli*, it was observed that CsgB, in addition to its role of nucleator, is also part of the fimbriae with the CsgA subunits. A structurally different fibril made of CsgB subunits can be formed in the absence of CsgA [40]. CsgA and CsgB share 30% of sequence identity and have the same predicted length [37]. In *E. coli*, interbacterial complementation between a nucleator mutant and a subunit mutant is possible. However, in *Salmonella*, this complementation cannot happen, suggesting that the curli fimbriae are different in their nucleation process. However, the interbacterial complementation was observed in *Salmonella* when a lipopolysaccharide O-antigen mutant was used [41]. The nucleation/precipitation pathway is still poorly understood, and research is actually performed on the different aspects of the curli fimbrial formation.
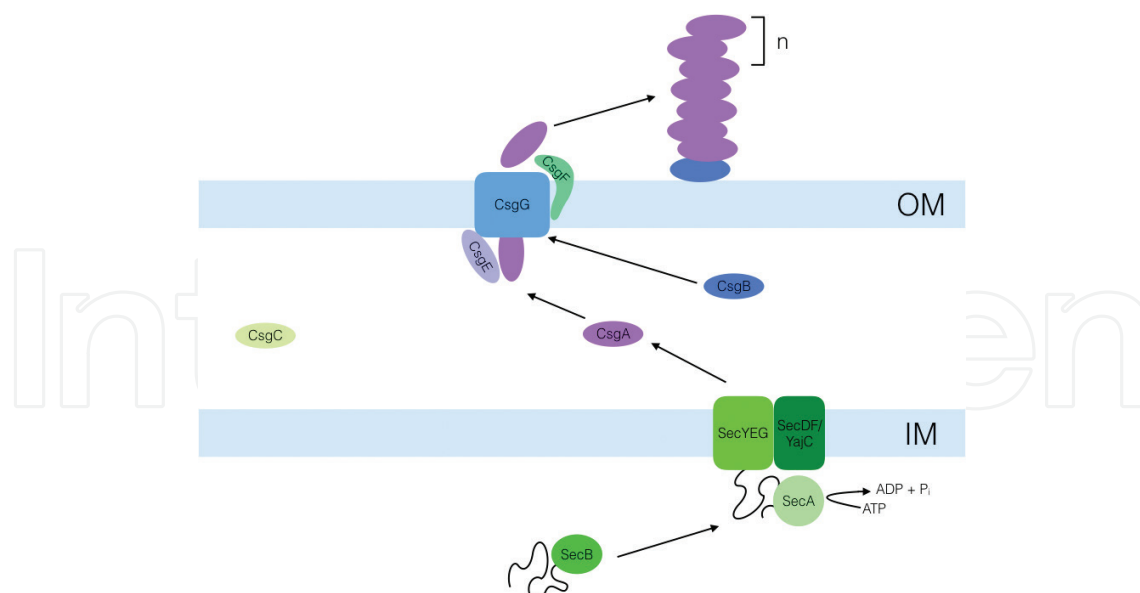
Figure 2. Nucleation/precipitation pathway. The subunit CsgA is synthesised in the cytoplasm and translocated by the GSP. CsgA passes through the periplasm and is translocated in the extracellular medium by CsgG, helped by CsgE. The nucleator CsgB is also translocated by CsgG and supported by CsgF for its stability on the bacterial surface. When CsgA is in the presence of the nucleator in the extracellular environment, the subunits precipitate in an aggregated fibril. CsgC is an oxidoreductase, but its specific role is still undiscovered. IM, inner membrane; OM, outer membrane.

2.3. Type IV fimbriae

Type IV fimbriae are usually from 1 to 5 μm long and are composed of repeated subunits of a single pilin. Type IV fimbria is subdivided into two groups based on homology of the major subunits: type IVa and type IVb fimbriae [26]. The difference between the two types is in the length of the peptide sequence and the mature major pilin sequence. Specific mechanism of assembly of type IVb fimbriae from *Salmonella* has not been characterised yet [42].

Type IV fimbriae pathway has the most complex machinery. They form an apparatus, composed of various proteins, that goes through the inner and outer membranes allowing the anchor of the fibre and energy accessibility for fimbrial assembly. The gene cluster also encodes numerous proteins with diverse functions, as the fibril is not only assembled but also disassembled. Type IV fimbriae are frequently compared to the type II secretion system (T2SS) which possesses similar structure and mechanism of assembly. Type IV fimbriae are implicated in adherence and twitching motility [11].

Type IV fimbriae are present in a variety of organisms including human pathogens such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. For *Salmonella*, they are found in *S. bongori*, *S. enterica* serovars Heidelberg, Paratyphi B and Typhi [42]. *S. bongori* type IV fimbria is encoded by the *sbe* operon that remains uncharacterised and is located on a plasmid, as well as in *S.* Paratyphi B, while the type IVb gene cluster is located on the chromosome of *S.* Heidelberg and *S.* Typhi [26].

For *S.* Typhi, the PilS subunits are produced in the cytoplasm and translocated to the periplasm by the GSP. In the periplasm, the N-terminal sequence of PilS is cleaved by PilU, a prepilin pep-

tidase [23]. The mature pilins are then anchored to the inner membrane on platform proteins and linked together into a fibril (**Figure 3**) [26, 43]. The N-terminal domain of the mature sub-units is highly hydrophobic, which permits the PilS proteins to group into a helical structure [22, 42]. The pilins are added one by one, but at three sites simultaneously, each corresponding to a strand to form a three-helix bundle [44]. An ATPase inserted in the inner membrane sup-plies the energy required for the assembly of the type IV fimbriae. The secretin proteins are inserted in the outer membrane and form a channel that permits the passage of the intact pilus through the bacterial surface [26]. These proteins form complexes that are then assembled in a cage-like final structure [44]. Other proteins are also involved in the assembly/disassembly mechanisms, such as another ATPase dedicated for the disassembly of the fimbriae, lipopro-teins of the secretin complex (pilotins), inner membrane proteins or gene products involved in peptidoglycan remodelling to permit the passage of the fibril through the periplasm [22, 44, 45]. This assembly pathway is less understood and requires further investigations [44].
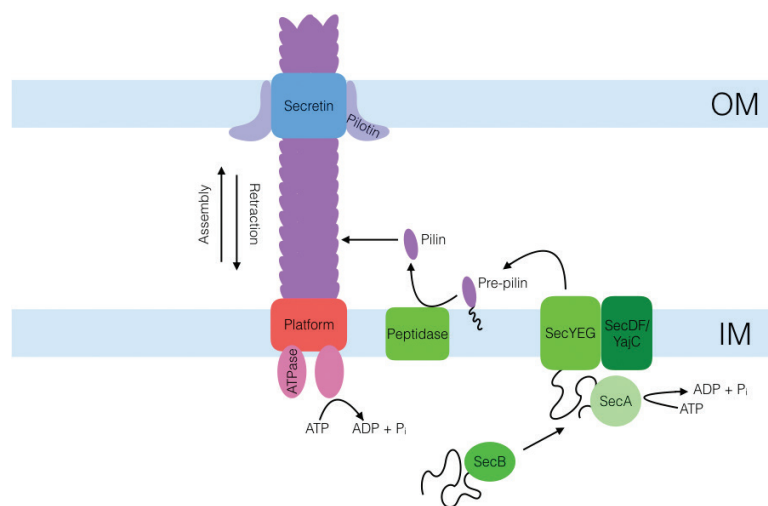
Figure 3. Type IV pathway. The pre-pilins are transported and translocated through the inner membrane (IM) to the periplasm by GSP. A peptidase cleaves the signal peptide of the pre-pilin, and the pilin can be assembled on the platform proteins. An ATPase triggers the reaction. The pilins form a three-helix structure that passes through the outer membrane (OM) by a secretin supported by pilotin. The type IV fimbriae can also retract depending on the environmental conditions.

3. *Salmonella* fimbriome

Each fimbrial pathway described above is present in *Salmonella* creating a great element of genetic diversity. CU fimbriae are the most common fimbriae detected in the *Salmonella* genome. Curli (*csg*) is found in all *Salmonella* genome, whereas only a few serovars have the type IV fimbriae. There are 38 unique FGCs identified so far in 111 sequenced genomes from 34 different serovars (**Table 1**) [46, 47]. Each serovar has its own repertoire of FGCs, but there are seven FGCs that are highly conserved in most *Salmonella* strains forming the core of *Salmonella* FGCs. Most of the FGCs are sporadic or found only in a few strains constituting the signature of each serovar.

Fimbriae	CU clade	Prevalence	Distribution	Fimbriae	CU clade	Prevalence	Distribution
bcf	γ1	Core	Absent in IV	*sdj*	γ4	Sporadic	IIIb *diarizonae*
csg	curli	Core	All *Salmonella*	*sdk/sfi*	π	Sporadic	IIIb, VI
fim	γ1	Core	Absent in *bongori*	*sdl*	π	Sporadic	IIIb *diarizonae*
lpf	γ1	Conserved	Absent in ID	*sef*	γ3	Sporadic	IB, D (pseudo)
mrk	γ4	Sporadic	Only in Montevideo	*sib*	β	Sporadic	VI *indica*
pef	κ	Sporadic	Only in IA, IC and *bongori*	*fae/skf*	κ	Sporadic	IB, IE
peg	γ4	Conserved	IB, IC, IIIa, VI, *bongori*	*ssf*	γ4	Sporadic	II *salamae*
peh	γ4	Sporadic	Only in Montevideo	*sta*	γ4	Sporadic	ID
pil		Sporadic	Type IV; ID, IE, *bongori*	*stb*	γ4	Core	I, II, IIIb;
saf	γ3	Conserved	ssp. I	*stc*	γ4	Conserved	IA, IB, ID
sba	γ4	Sporadic	*bongori*	*std*	π	Core	II, IIIa, missing in Gallinarum
sbb/sbf	π	Sporadic	*bongori*	*ste*	π	Conserved	Missing in IA, IE
sbc/spf	κ	Sporadic	IV, VI, *bongori*	*stf*	π	Conserved	Missing in ID, IE
sbs	β	Sporadic	II *salamae*	*stg*	γ1	Sporadic	ID, bongori
sdc/sas	σ	Sporadic	IIIa *arizonae*	*sth*	γ1	Core	Missing IIIa and IIIb
sdd/smf	γ1	Sporadic	IE, II, IIIa, IV	*sti*	γ1	Conserved	Missing in ID
sde	γ3	Sporadic	Tennessee (IE)	*stj*	β	Sporadic	IA, IE
sdh	γ4	Sporadic	IE	*stk*	γ4	Sporadic	IE
sdi/sdf	γ4	Sporadic	IIIb *diarizonae*	*tcf*	α	Sporadic	IC, ID, IE

Table 1. *Salmonella* fimbriome.

Each *Salmonella* strain contains 5–14 different CU fimbriae with an average of 12 fimbriae in *S. enterica*. Representatives from all the six phylogenetic clades are present in *Salmonella* (**Table 2**) [26]. The γ-fimbriae constitute the largest clade with 22 FGCs and include the highly conserved FGCs (*bcf, fim* and *sth*) that belong to the clade γ-1. The most diverse clade is γ-4, with the conserved *stb* and *stc* or *peg* (*stc-peg*) and many of the new sporadic FGCs, while the α clade (for alternate CU), also known as class 5 fimbriae, has one FGC, *tcf*, which is found in several serovars. The σ clade also had only one FGC representative, *sdc*, that was only found in *S. enterica* subspecies IIIa (*arizonae*).

The distribution of the 38 FGCs gave a signature for each species, subspecies and serovars (**Table 3**). Seven FGCs, curli and the CU *fim, bcf, sth, stb, stc-peg* and *std*, represent the conserved (core) fimbriae of *Salmonella* (positive in more than 90% of strains). The *fim* fimbriae were found in all *S. enterica* strains, only missing in *S. bongori*. The *bcf* cluster was only missing in *S. enterica* ssp. IV (*houtenae*), and the *sth* cluster was only missing in *S. enterica* ssp. IIIa and IIIb. The *stb* cluster was present in *S. enterica* ssp. I, II, IIIb and the *std* cluster was not detected in *S. enterica* serovar Gallinarum, ssp. II, IIIA and *S. bongori*. The FGC *stc* and *peg* had probably emerged from a common ancestor: they belong to the same clade (γ-4) and are inserted at the same position in the genome (between *thiM* and *mrp*); their distribution is mutually exclusive; and either one is present in the majority of *Salmonella* strains.

CU clade	Fimbriae
α	*tcf*
β	*sbs, sib, stj*
γ1	*bcf, fim, lpf, sdd/smf, stg, sth, sti*
γ3	*saf, sde, sef*
γ4	*mrk, peg, peh, sba, sdh, sdi, sdj, ssf, sta, stb, stc, stk*
κ	*fae/skf, pef, sbc/spf*
π	*sbb/sbf, sdk, sdl, std, ste, stf*
σ	*sdc/sas*

Table 2. *Salmonella* fimbrial type.

Most cases of salmonellosis in humans are caused by *S. enterica* ssp. I, and many of the sequenced serovars were from ssp. I. Thus, 27 out of the 38 FGCs are found in ssp. I. The ssp. I was divided into five classes using previous phylogenetic analysis [46, 47] (**Table 3**). The class IA contains broad host range serovars involved in gastroenteritis, mainly serovar Typhimurium. The class IB is formed by serovars Dublin, Enteritidis, Pullorum and Gallinarum, all sharing similar O-antigens and FGCs. The class IC contains serovars Choleraesuis and Paratyphi C and class ID contains the human-specific serovars Typhi and Paratyphi A. A separate branch of class IA, including serovars Heidelberg, Virchow and Hadar, that had the highest number of FGCs, as well as serovars Montevideo, Schwarzengrund, Welterveden, Javiana, Kentucky and Tennessee, was commonly isolated in association with edible plants and constitutes the class IE.

In addition to the seven core FGCs, five highly conserved FGCs (*saf, ste, stf, sti* and *lpf*) were associated with *S. enterica* ssp. I (**Table 3**). The *sti, lpf* and *stf* clusters are missing in human-specific serovars (class ID). The *ste* cluster is missing in class IA serovars and in some of the class IE serovars. Thus, *S. enterica* ssp. I harbours the core FGCs (*fim, bcf, sth, stb, stc-peg* and *std*), the conserved FGCs (*saf, ste, stf, sti* and *lpf*) and some sporadic FGCs unique to each serovar. Many FGCs of *Salmonella* are sporadic and form the unique repertoire in each serovar.

Despite the presence of many FGCs, extensive gene degradation was observed in most of the host-restricted and warm-blooded host-adapted serovars, mainly Gallinarum, Choleraesuis,

Paratyphi A and Typhi. Genome degradation of FGCs may correspond to the loss of genes rendered unnecessary by niche specialisation or by selective pressure in order to diminish antigen presentation at the bacterial surface during systemic disease. Intriguingly, most of FGCs were intact in Paratyphi B.

There are 11 FGCs that are not in ssp. I, with only *sbc* and *sdk* that are shared by more than one serovars. The low numbers of FGCs might be specific for cold-blooded animals' colonisation. A conserved signature specific for each subspecies was observed. As more diverse strains will be sequenced, new FGCs probably be discovered.

	Subspecies	Core	Conserved		Accessory	Absent
Salmonella enterica	I. *enterica*	*bcf, csg, fim, sth, stb, std* *stc-peg*	*sti,, saf, ste, stf, lpf*	A	*pef, stj*	*ste*
				B	*fae, sef*	
				C	*pef, tcf,*	
				D	*sef, sta, stg, tcf, pil*	*sti, lpf, stf*
				E	*fae, mrk, peh, sdd, sde, sdh, stj, stk, pil*	*lpf, ste, stf*
	VI. *indica*				*sbc, sdk, sib*	*stb*
	II. *salamae*				*sdd, ssf, sbs*	*std*
	IV. *houtenae*				*sbc, sdd*	*bcf, stb*
	IIIb. *diarizonae*				*sdi, sdj, sdk, sdl*	*sth*
	IIIa. *arizonae*				*sdc, sdd*	*sth, stb, std*
Salmonella bongori			*lpf*		*sba, sbb, sbc, sbe, stg(sbd)*	*fim, stb, std*

Table 3. Fimbrial distribution.

4. Fimbrial regulation

Salmonella fimbriae are usually not expressed constitutively and rarely expressed under laboratory condition, except for Fim fimbriae, a type-1 fimbria [3]. Fimbriae are important during infection [19, 48, 49], suggesting that their expression is tightly regulated. Little is known about the regulation mechanisms that promote fimbrial expression. In general, fimbrial expression is positively or negatively regulated at the genetic level. Some regulators are unique to a specific fimbriae, like the regulation of curli by CsgD, while others are global, like Dam, H-NS and Lrp (leucine-responsive regulatory protein) [50]. These mechanisms include regulatory proteins, DNA methylation, cyclic di-GMP and small RNAs [50]. In *Salmonella*, a regulation network exists between the virulence factors. Here, we present the regulation of fimbrial genes including the interaction with motility and invasion. Then, we propose an example of regulation of the *fim* FGC expression in *S*. Typhimurium, the most characterised fimbriae of *Salmonella*.

4.1. General regulation of fimbrial genes

Genes implicated in different aspects of virulence including motility, adhesion, invasion of host cells and intestinal persistence are all regulated during infection. It was proposed that there is a temporal hierarchy between the T3SS of SPI-1 (invasion), flagellar and fimbrial genes, where SPI-1 is first activated, followed by flagellar genes and then type-1 fimbrial genes (*fim*). The crosstalk between these systems seems to be critical for bacterial pathogenesis [51]. Each element of virulence is related to a large regulation network that is not completely understood. DNA adenine methylation (Dam) regulates many virulence genes in *Salmonella* [52]: it is required for SPI-1 and *pef* expression, but it also represses many genes, including the *std*, *csg* and flagellar genes [52–54]. It was also shown that fimbrial FGCs are repressed by the Rcs phosphorelay, a sensor of outer membrane stress [55]. Another example of regulation interaction between motility and fimbrial expression was observed by a deletion of *ydiV* in *S.* Typhimurium that results in the derepression of curli fimbriae (*csgAB*), causing an increase in swimming motility and a decrease in swarming [56].

Crosstalk regulation also occurs between the capsule and the type IVb fimbriae in *S.* Typhi. Both virulence factors are encoded on SPI-7 and facilitate invasion of monocytes, suggesting a regulation overlapped. However, the exact regulation elements that act on those two systems are unknown [57].

One of the post-transcriptional regulation mechanisms uses the binding of small RNAs and the Hfq chaperone. In an *hfq* mutant strain, the expression of fimbrial gene *sefA* was activated when most of the other fimbrial subunit genes were repressed in *S.* Enteritidis. Overall, the *hfq* deletion decreased adherence compared to wild-type strain. Thus, Hfq seems to regulate fimbrial expression of most fimbrial genes from *S.* Enteritidis [58]. There is probably more sRNAs regulation of fimbrial gene expression awaiting to be discovered.

Phase variation is a transcriptional mechanism that controls the switch between fimbriated (ON) and afimbriated (OFF) cells within a bacterial population. In *Salmonella*, expression of *lpf* and *pef* was shown to be controlled by phase variation. The regulators of this mechanism are various and depend on the FGCs concerned [54, 59].

The secondary messenger cyclic-di-GMP controls virulence and biofilm formation in *Salmonella* [60]. In *Salmonella*, curli expression was activated by AdrA, a GGDEF-domain protein that increases intracellular level of cyclic-di-GMP [61]. Fimbrial production regulated by the cyclic-di-GMP level was also observed in other species such as *Klebsiella pneumonia*, *E. coli* and *P. aeruginosa* [62].

In spite of all those known elements of regulation, how *Salmonella* passes from being afimbriated in vitro to a fimbriated form in vivo is still unknown.

4.2. Regulation of *fim* in *S.* Typhimurium

The *fim* FGC codes for six genes (*fimAICDHF*). This cluster is the most studied and one of the most conserved fimbriae of *Salmonella enterica* and was mainly characterised in *S.* Typhimurium. These fimbriae have a binding specificity for mannose residues [63]. The fim fimbria of *Salmonella* is not homologue with its homonym from *E. coli*, except for sharing some morphological and

mechanistic features [64, 65]. Regulation and amino acid sequences of fimbrial proteins are divergent between the two species. The transition from afimbriated to fimbriated stage occurs for *fim*, but there is no inversion of the promoter region as observed for *E. coli* phase variation [66]. For *S.* Typhimurium, the major subunit FimA is accompanied by two other subunits, FimI and FimF, and by the adhesin, FimH. The *fimC* and *fimD* genes encode respectively the chaperone and the usher [67]. Ancillary genes, *fimZYW*, and a rare arginine transfer RNA (tRNA) *fimU* and *STM0551*, inserted between *fimY* and *fimW*, directly regulate *fim* expression [62].

Ancillary genes *fimZYW* regulate the expression of *fimA* [50, 68–70]. FimZ, a sensor DNA-binding protein, is the principal positive regulator of *fimA* [71]. FimY upregulates *fimZ* expression by binding to the *fimZ* promoter. FimY and FimZ then form a complex that activates the *fimA* promoter [63, 69]; *fimY* is itself regulated by the arginine tRNA *fimU* [72]. Lrp is another regulator that binds and activates the *fimZ* promoter, probably by antagonising the binding of the global repressor protein H-NS to this promoter region [67, 70, 73]. H-NS has a high affinity for AT-rich DNA region, and *fimZ* gene has an unusual AT-rich sequence [73].

At the opposite, FimW repressed directly *fimA* expression and indirectly by lowering FimZ availability by its degradation caused by FimW binding [74]. It is also suggested that STM0551, an EAL domain protein, is a negative regulator of *fim* expression by lowering the c-di-GMP level [62].

FimZ is also used as a regulator relay by two-component system for expression of *hilA*, the principal regulator of SPI-1 (invasion). The two-component system PhoBR induces *fimZ* expression, and PhoPQ activates *hilE*, one of the negative regulators of *hilA*, by a FimYZ-dependant manner. FimZ also downregulates *flhDC*, genes implicated in flagella expression [75]. Flagellar gene *fliZ* also represses *fimZ* on a post-transcriptional manner, reinforcing the fact that there is an alternated expression of flagella (motility) and fimbriae (adhesion) [51] and confirming a regulation network between SPI-1 (invasion) and fimbrial expression [76]. Thus, a combination of factors directly implicated in fimbrial genes regulation can also impact on other virulence systems of *Salmonella* (**Figure 4**).

Figure 4. *fim* regulation. The fim FGC is activated and inhibited by diverse regulators. FimZ, accompanied by FimY, is the principal activator of *fimA*. Lrp and c-di-GMP also activate *fimA* at the promoter level. H-NS and FimW inhibit *fimA* expression by linking its promoter. FimW also reduces *fimA* expression by linking FimZ and decreases the availability of this activator. The gene *fimZ* is activated by FimZ itself and by FimY, but is repressed by FliZ in a post-transcriptional manner. The tRNA *fimU* regulates fimY. FimZ downregulates *flhCD*, genes implicated in flagellar expression.

5. Fimbriae as a tool

Salmonella infections are a major concern for public and animal health. Some serovars are host specific, while others are broad-spectrum pathogens and can be transmitted from food-borne animals to humans. On the other hand, animals can develop health problems and will not be suitable for consumption. To prevent those issues, it is critical to develop ways to detect *Salmonella* and protect potential hosts against infection. The importance of fimbriae for detection of *Salmonella* by molecular techniques and for vaccine development is presented in this section [77].

5.1. *Salmonella* detection using fimbrial genes

Salmonella-specific tests were performed since the end of the 1980s and mainly targeted surface antigens. Those tests include agglutination tests and ELISA (enzyme-linked immunosorbent assays) [77–79]. In 1993, Doran et al. presented a DNA-based test that targets *csgA* (*agfA*), offering a faster and more precise test for genus identification [80]. Then, in early 2000s, PCR (polymerisation chain reaction) tests using fimbrial genes, like *sef* or *csgA* (*agfA*), in combination with other virulence genes were developed to differentiate *Salmonella* strains from each other [81]. Different PCR tests (multiplex, nested and direct PCR) were elaborated for detection of *Salmonella*. Several of those tests integrated detection of fimbrial genes (i.e. *staA*, *fimW*) to discriminate between serovars [81–83]. Recently, a loop-mediated isothermal amplification (LAMP) assay was developed to detect *Salmonella* by targeting *bcfD*, a gene that belongs to the core of FGC. In isothermal conditions, the reaction occurs in an hour permitting rapid detection of *Salmonella* [84].

Salmonella-specific tests evolved from detecting antigens, which can be long and expensive to perform, to detecting specific genes in less than an hour by sensitive methods. Fimbrial genes are tools of choice for detection of *Salmonella*. The presence of conserved fimbrial genes allows the discrimination between *Salmonella* and non-*Salmonella* species. On the other hand, the presence of a specific pattern of fimbrial genes enables the discrimination between serovars.

5.2. Vaccines development

As surface structures, fimbriae constitute antigens of choice for the development of vaccines against *Salmonella* [85]. Fimbriae are difficult to study because they are poorly expressed under laboratory conditions and are redundant. The most interesting fimbriae are the ones expressed during infection. Targeting those fimbriae will confer higher chances to be recognised by the immune system in key moments of infection.

More than 20 fimbrial antigens were detected in typhoid fever patient's blood by transcriptomic analysis: SteD, StaACD, BcfDE, SafBC, TcfBCD, StbBC, FimAIDH, StdBC, StgACD and SthA [86]. Antibodies against immunogenic fimbrial proteins TcfB, StbD and CsgEFG were identified in the blood of typhoid fever patients [12]. Immunoreactive antibodies against SthDA and BcfA were found in lymphocytes supernatant (ALS) of patients with typhoid fever [87].

SefA, a protein from the SEF14 fimbriae of *S.* Enteritidis, was used as an antigen associated with liposomes for oral immunisation of chickens [88]. The immunisation of chickens by fimbrial antigens was efficient for IgG and IgA responses and reduced *Salmonella* colonisation. Four weeks after immunisation, the bacterial excretion from the intestinal tract was significantly reduced [88]. The liposome-associated immunisation was also performed with fimbrial antigen from SEF21 and resulted in a similar efficiency [89]. SefD, another antigen from SEF14, was also used to vaccinate animals in a bacterin preparation, a vaccine prepared from inactivated bacteria. This vaccine was efficient to reduce the presence of *Salmonella* from the spleens of hens [90].

As factors implicated in the first stages of infection, fimbriae are an interesting target for vaccine development [91]. Fimbrial antigens are important for the development of new anti-*Salmonella* therapies [85, 86]. However, a better understanding of their expression pattern in vivo is needed to optimise the therapeutic effects of fimbrial-targeted vaccines. Fimbrial antigens may be combined with other immunogenic proteins to increase the immune response [91].

6. Conclusion

Fimbriae are diverse proteinaceous surface structures. They diverge by their assembly mechanisms and result in different filamentous structures with roles in pathogenesis. However, their roles are not completely understood. They were first known for adherence to cells and inert surfaces, but they seem to be implicated in so much more functions during infection.

The multiplicity of adhesion systems is also an enigma. Most of the *Salmonella* serovars possess 12 fimbrial gene clusters. Some fimbriae are specific to certain serovars and may play a role in these bacteria that do not need to be fulfilled in other serovars. At the opposite, there is a core of fimbrial genes that are present in most of the serovars. Fimbriae are one of the keys to understand *Salmonella* pathogenesis. The specific pattern of each serovar, with further investigations on the sporadic fimbriae, may also bring insights into our understanding of *Salmonella* pathogenesis.

Regulation of fimbrial genes is a complex network that is tightly related to invasion and motility. Virulence factors are finely regulated, and a temporal expression hierarchy allows the success of *Salmonella* infection. General regulators are already known to regulate fimbrial genes such as stress sensor Rcs relay or the Hfq factor. Phase variation from a fimbriated to afimbriated status occurs in *Salmonella*. However, this phenomenon is not from a promoter inversion of the Fim cluster, but from the regulation by ancillary genes related to *fim* gene cluster. These ancillary genes are themselves precisely regulated by a variety of regulators known for their role in other bacterial processes.

The actual understanding of fimbrial expression opens a new area on human health prevention. Some conserved fimbrial genes, in combination with other virulence genes, are precious markers for *Salmonella* detection. These tools could permit a faster diagnostic for human patients, but also a rapid detection of contaminated food or infected animals. Fimbrial proteins can serve as good immunogens in vaccine preparation against *Salmonella* infection.

A better understanding of fimbrial expression, production and regulation processes becomes important for prevention of *Salmonella* infection. It will also enlighten the importance of fimbriae in other human pathogens, as fimbrial systems are part of virulence factors in many bacteria.

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