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# Molecular Armory of *S. Typhi*: Deciphering the Putative Arsenal of Our Enemy

Chantal G. Forest and France Daigle

*Department of Microbiology & Immunology/University of Montreal  
Canada*

## 1. Introduction

The outer surface of bacteria is the first to interact with host components, such as the immune system, the extracellular matrix or cells. The bacterial Gram-negative cell wall is complex and composed of an inner membrane (IM), a periplasmic space and a thin peptidoglycan layer, all surrounded by an outer membrane (OM). The OM is a bilayered structure consisting mainly of phospholipids, proteins and lipopolysaccharide (LPS) and serves as an impermeable barrier to prevent the escape of periplasmic molecules but also acts as a barrier for entry of external molecules. *Salmonella enterica* comprises more than 2500 serovars, based on three major antigens located at the cell surface: O antigen, capsule and flagella. All serovars are highly conserved genetically but have different host ranges and cause different diseases. In humans, *Salmonella* infection causes gastroenteritis, often associated with serovars Typhimurium and Enteritidis or typhoid-like disease, which is associated with serovars Typhi and Paratyphi. *S. Typhi* strains belong to serogroup D1 with the antigenic formula O:9,12; Vi+; H-d. These strains are human-restricted and besides asymptomatic carriers, no environmental reservoir is known.

*S. Typhi* is a monomorphic bacterium, showing very little genetic diversity (Kidgell et al., 2002) and up to 5% of its annotated coding sequences are pseudogenes (Holt et al., 2009; Parkhill et al., 2001). Genome degradation may be responsible for its host specificity; however the *S. Typhi* genome may harbour specific genes for its systemic dispersion and survival. *S. Typhi* remains a major public health problem in developing countries. Antimicrobial resistance has become a problem in endemic regions, and it is becoming imperative to develop new vaccine strategies or discover new antimicrobial targets to combat this microorganism. Bacterial surface proteins may correspond to these targets by being immunogenic or essential for virulence. Most virulence factors are usually located within genomic locations called *Salmonella* Pathogenicity Islands (SPIs) and are tightly regulated by global regulators such as PhoP-PhoQ, RcsDBC, OmpR-EnvZ and RpoS. This review will focus on molecules localized at the outer membrane of *S. Typhi* and their role in pathogenesis. A complete analysis of adhesive molecules, such as the 12 fimbrial systems, curli, type IVB pilus, autotransporters and afimbrial adhesins will be presented. We will also discuss the importance of polysaccharides such as the Vi capsule and LPS. Furthermore, the complex surface structures generated by secretion systems, such as type three secretion systems (T3SS), flagella and T6SS that are so important for invasion, intracellular survival and to hijack the host defence system will be discussed. Finally, methods used to inhibit these adhesive structures will be described.

2. Fimbrial adhesins

Fimbriae (also called pili) are proteinaceous structures that can be observed as filaments anchored on the bacterial cell surface. These structures can mediate crucial interactions during host infection like adherence, invasion or biofilm formation, and are classified according to their mechanism of assembly. Most of the fimbriae present in *S. Typhi* genome are assembled by the chaperone/usher pathway, but there are also one representative of the nucleation/precipitation pathway (*csg*) and one type IVB pilus. This section will briefly describe each mechanism of expression and the current knowledge related to *S. Typhi* and their putative roles.

2.1 Mechanisms of fimbrial assembly

2.1.1 The chaperone/usher pathway

Twelve fimbrial systems detected in *S. Typhi* belong to the chaperone/usher pathway (CUP) assembly class (Fig. 1). A classic fimbrial operon usually harbours at least four different genes. The filaments are composed of major and minor fimbrial subunits assembled by the cooperative work of the chaperone and the usher. After translocation by

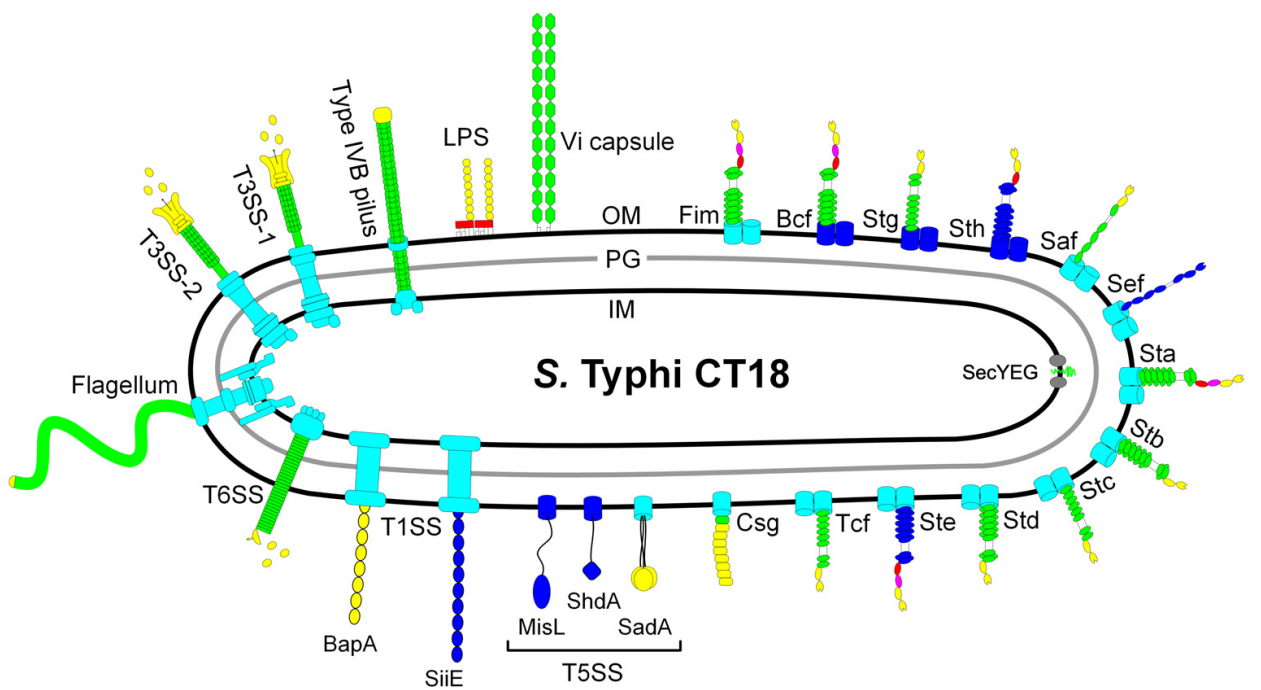


Fig. 1. Schematic representation of the important surface structures identified in *S. Typhi* CT18. Adhesive components are shown in yellow, membrane components are cyan and pseudogenes are shown in blue (pseudogenes of the T6SS are not shown). The twelve representatives of the CUP are grouped in fimbrial clades and are drawn according to previous observations (Salih et al., 2008) or based on their homologues found in *E. coli* K-12 (Korea et al., 2011). STY0405 putative autotransporter, STY0351 adhesin, and PagC which are known to be implicated in virulence were omitted from the drawing, as well as STY1980 (MAM7). IM stands for inner membrane, PG for peptidoglycan and OM for outer membrane.

the Sec general secretory pathway, the periplasmic chaperone protects the subunits and brings them to the OM usher, which specifically translocates subunits to the cell surface. Fimbrial biogenesis by the CUP pathway is a self-energized process catalyzed by both the usher and the presence of high-energy intermediates in the folding of the chaperone-subunit complexes (Jacob-Dubuisson et al., 1994; Nishiyama et al., 2008; Sauer et al., 2002; Zavialov et al., 2002). Classification based upon sequence homology between the different ushers (Nuccio & Bäumler, 2007) revealed members in the  $\gamma$ 1- (*bcf*, *fim*, *stg*, *sth*),  $\gamma$ 3- (*saf*, *sef*),  $\gamma$ 4- (*sta*, *stb*, *stc*),  $\Pi$ - (*std*, *ste*) and the  $\alpha$ -fimbrial clades (*tcf*) in *S. Typhi* (Fig. 1).

### 2.1.2 Nucleation/precipitation pathway

The thin aggregative fimbriae, also known as curli or TAFI, encoded by the *csgDEFG csgABC* gene cluster belongs to this class of adhesin. The first steps of biogenesis are similar to the CUP : after translocation by the Sec pathway, CsgA and CsgB fimbrial subunits are secreted by the CsgG outer membrane protein at the bacterial cell surface. The major difference between curli and CUP lies in its extracellular fiber growth assembly (Hammar et al., 1996). After secretion of the CsgB subunit, CsgA precipitates, polymerizes on CsgB and adopts an insoluble structure related to amyloid fibers (Hammar et al., 1996).

### 2.1.3 Type IVB pili

One of the most studied adhesive structures of *S. Typhi* is the type IVB pilus encoded by the *pil* operon located on SPI-7. Although type IV pili also produce long and flexible structures on the bacterial cell surface, their mechanism of assembly strongly differs from the CUP and curli pathways as it requires many structural proteins and is an ATP-dependent process. First, PilS prepilins are translocated through the IM into the periplasm and a specific prepilin peptidase cleaves the N-terminal signal peptide (reviewed in Craig & Li, 2008). An integral IM protein mobilizes a specific ATPase from the cytoplasm which drives pilus assembly. An oligomeric channel called the secretin found in the OM allows the exit of the pilus at the cell surface of the bacteria. ATP hydrolysis moves the pilus out in the secretin pore allowing the recruitment of new prepilin subunits. Unlike CUP and Csg fimbriae, Type IV pili are still connected to the IM of the bacteria and can be retracted rapidly inside the bacteria.

## 2.2 Roles of fimbrial adhesins during typhoid fever

In most studies, *Salmonella* fimbriae are involved during intestinal colonization (Althouse et al., 2003; Chessa et al., 2009; Weening et al., 2005), or in biofilm formation (Boddicker et al., 2002; Ledeböer et al., 2006), although they can also be used during the systemic phase (Edwards et al., 2000; Lawley et al., 2006). Interestingly, each serovar of *Salmonella enterica* harbours a unique combination of fimbrial operons, probably to avoid cross-immunity between two serovars infecting the same host (Norris & Bäumler, 1999; Nuccio et al., 2011). As *S. Typhi* infects only humans, little is known regarding the conditions of expression or the implication of each fimbrial adhesin during the course of infection. While some clues may be found in the literature, there is still much work to be done. Three fimbrial systems are clustered within pathogenic islands: *tcf* (Typhi colonizing factor) and *saf* (*Salmonella* atypical fimbriae) are found within SPI-6, while *sef* is in SPI-10 (Sabbagh et al., 2010).

Proteins expressed during infection were detected in blood of patients with typhoid fever (Charles et al., 2010; Harris et al., 2006; Hu et al., 2009). Interestingly, six proteins related to fimbrial adhesins led to the formation of antibodies after typhoid fever (TcfB, StbD, CsgG, CsgF, CsgE and BcfD). Since three proteins belonging to the thin aggregative fimbriae were identified, it suggests a strong production *in vivo* as well as an important role during infection. Csg implication during attachment to surfaces, bacterial autoaggregation and in biofilm formation is well known for *S. Typhimurium* and *E. coli* (Jonas et al., 2007). Nevertheless, a clear characterization of *csg* is needed for *S. Typhi* as there seem to be variations in expression between the different isolates (Römling et al., 2003; White et al., 2006). In *S. Typhi*, a strong expression of *csg* and *saf* fimbrial operons was observed inside human macrophages (Faucher et al., 2006).

The *bcf*, *sef*, *ste*, *stg* and *sth* fimbrial systems harbour pseudogenes that might disrupt the production of the corresponding fimbriae (Townsend et al., 2001). However, deletion of *stg* leads to reduced adhesion on epithelial cells as well as enhancement of the phagocytosis rate by macrophages (Forest et al., 2007). Furthermore, the presence of antibodies directed against BcfD is intriguing since the *bcfC* usher harbours two premature stop codons (Parkhill et al., 2001). The Bcf, Stb, Stc, Std and Sth fimbrial systems are required for the intestinal persistence of *S. Typhimurium* in mice, but their roles during the pathogenesis of *S. Typhi* still need to be evaluated (Weening et al., 2005). Sta and Tcf do not seem to be used for adhesion or invasion of non-polarized human epithelial cells while both are expressed at high NaCl concentrations (Bishop et al., 2008). Since these two fimbriae are found almost exclusively in the genome of serovars causing typhoid fever, they might be involved during the systemic phase or for the chronic carrier state (Nuccio et al., 2011). Although roles for Saf (Carnell et al., 2007; Lawley et al., 2006), Sef (Edwards et al., 2000) and Std fimbriae (Chessa et al., 2008; Weening et al., 2005) have been observed in other serovars of *Salmonella*, their true implication during typhoid fever needs to be investigated.

Type 1 fimbriae encoded by the *fim* operon are the best studied fimbrial adhesins and are frequently found in enteric bacteria. Fim are characterized by their mannose-sensitive binding properties, but their cell tropism seems to vary greatly between species and even between different strains of the same serovar (Thankavel et al., 1999). In *S. Typhi*, most clinical strains are fimbriated (*fim*+) and afimbriated strains are less adhesive and invasive than the fimbriated ones (Duguid et al., 1966; Satta et al., 1993). The ability of type 1 fimbriae to agglutinate yeast is abolished when the Vi capsule is expressed (Miyake et al., 1998). In *S. Typhimurium*, Fim appears to be the only fimbrial adhesin expressed in Luria-Bertani (LB) broth as confirmed by electron microscopy and flow cytometry (Duguid et al., 1966; Humphries et al., 2003). In *S. Typhi*, a complete deletion of *fim* also showed no evident fimbrial structures on the cell surface of the bacteria after growth in LB broth (Fig. 2).

Type IVB pili interact with the cystic fibrosis transmembrane conductance regulator (CFTR), a receptor upregulated and actively used by *S. Typhi* for its interaction with human epithelial cells (Lyczak & Pier, 2002; Pier et al., 1998; Tsui et al., 2003). These pili can also mediate bacterial self-association in conditions found in the intestinal tract, probably by enhancing binding efficiency prior to cell invasion (Morris et al., 2003a; Morris et al., 2003b). A direct correlation was observed between the level of surface-exposed CFTR and the efficiency of invasion of *S. Typhi* through the intestinal barrier (Pier et al., 1998). This specific interaction can be blocked by the addition of prepilin pre-PilS in the cell culture medium or with monoclonal antibodies specific to the first extracellular domain of CFTR



(Pier et al., 1998; Zhang et al., 2000). A piliated strain also adheres and invades human monocytes in a greater extent than a non-piliated strain and its expression can also increase IL-6 and NF-kappa B production in human monocytes by activating protein kinase C (Pan et al., 2005; F. Wang et al., 2005). Only a few other serovars, such as *S. Paratyphi* B and C, *S. Heidelberg* and *S. bongori* possess the genetic information coding for type IVB pili (Nuccio et al., 2011). Other functions could potentially be found in future studies as Type IV pili are also implicated in a variety of processes like biofilm formation, immune escape, DNA uptake and phage transduction in other pathogenic bacteria (reviewed in Craig & Li, 2008). These pili can also act as pistons, retracting subunits into the bacteria while it is still attached to a surface in a mechanism called “twitching motility” providing flagella-independent motility (reviewed in Mattick, 2002).

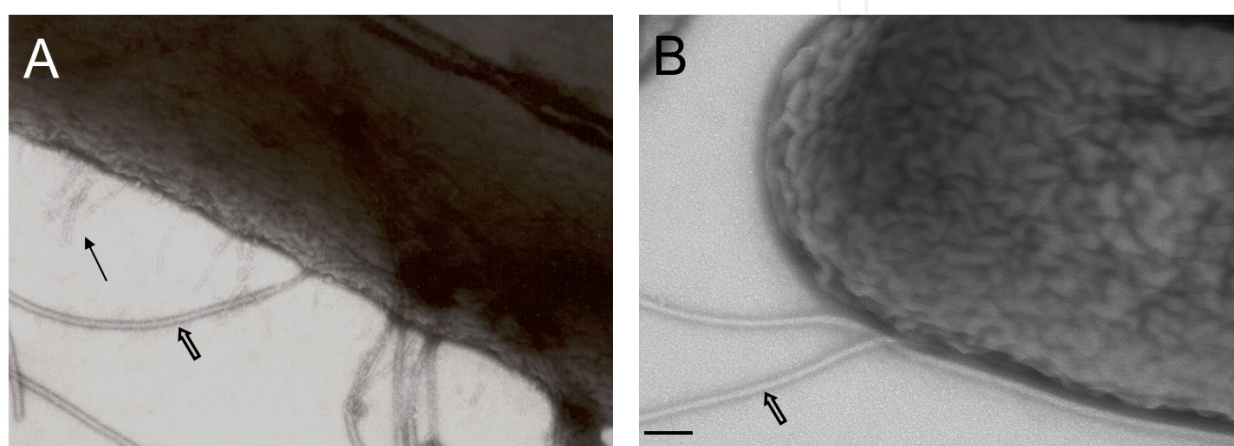


Fig. 2. Surface observation of *S. Typhi* grown in LB broth by transmission electron microscopy. After negative staining with phosphotungstate 1%, fimbriae were observed at the cell surface of the wild-type ISP1820 strain (A), while no structure was observed when *fim* was deleted (B). Black arrow shows fimbria and the open arrows indicate flagella. Black bar = 100 nm.

No genes related to fimbrial operons were found after a screening for mutants with a competitive disadvantage in humanized mice engrafted with hematopoietic stem cells (Libby et al., 2010). This result strongly suggests that fimbrial operons are mostly required during interaction with human epithelial cells, such as intestinal and gallbladder cells, that are absent from this mice model. Moreover, functional redundancy is often observed for fimbrial adhesins making it hard to evaluate their true contribution by single mutations. In order to understand the specific role played by each fimbrial system, our laboratory is currently creating a *S. Typhi* strain with deletions of all its fimbrial adhesins. This strain will greatly help to evaluate the global contribution of each fimbrial adhesins during association with eukaryotic cells.

### 3. Non fimbrial adhesins

#### 3.1 Type 1 secretion systems

In *Salmonella*, some important surface structures are expressed by different mechanisms and can be classified as non fimbrial adhesins. In *S. Typhi*, there are two examples of adhesins secreted by a type I secretion system (T1SS) : SiiE and BapA. In T1SS, the secreted proteins

directly pass through a channel formed between the IM and OM of the bacteria by the recognition of a signal at the C-terminus (China & Goffaux, 1999 as cited in Main-Hester et al., 2008). SPI-4, present in all *Salmonella* strains, encodes a T1SS responsible for the secretion of SiiE, the largest protein found in *Salmonella* (595 kDa) (Latasa et al., 2005; Main-Hester et al., 2008). Its cell surface expression requires the IM ATPase SiiF, the periplasmic adaptor SiiD and an outer membrane channel formed by SiiC (Gerlach et al., 2007). This adhesion system acts in a coordinated way with the T3SS of SPI-1 and is involved during the intestinal phase of infection (Gerlach et al., 2008). Previously annotated as two distinct ORFs in *S. Typhi* (STY4458-4459) (Parkhill et al., 2001), *siiE* harbours a premature stop codon probably rendering this large adhesin non-functional (Main-Hester et al., 2008; Morgan et al., 2004). An immunoblot done with antibodies directed against SiiE (STY4458) demonstrated the absence of production of SiiE in the whole cell proteins of *S. Typhi* further suggesting a lack of function in this serovar (Main-Hester et al., 2008). However, a transposon insertion in STY4458 showed a reduced competitive fitness in humanized mice, suggesting SiiE functionality and an uncharacterized role during interaction with hematopoietic cells (Libby et al., 2010).

A second T1SS is clustered within SPI-9 and secretes another large repetitive protein called BapA (**biofilm-associated protein**) due to its similarity with BapA of *Staphylococcus aureus*. Well described in *S. Enteritidis*, BapA is involved in bacterial autoaggregation strongly inducing biofilm formation and is also required during the interaction with the intestinal mucosa (Latasa et al., 2005). Its expression is under the control of CsgD, an important regulator also coordinating curli fimbriae and cellulose production needed for biofilm production (Jonas et al., 2007). Again, solving the components required for biofilm formation by *S. Typhi* is crucial since *bcsC* (STY4184), essential for cellulose and biofilm production, is a pseudogene (Parkhill et al., 2001; Zogaj et al., 2001).

### 3.2 Type 5 secretion systems

Autotransported adhesins can be monomeric or trimeric and are considered as type 5 secretion systems. *S. Typhi* harbours two monomeric examples of autotransported adhesins, *shdA* (STY2755) found in the CS54 island and *misL* (STY4030) clustered in SPI-3, as well as one representative of a trimeric autotransporter called *sadA* (STY4105). An N-terminal signal sequence allows their translocation into the periplasm by the Sec general secretory pathway, then a  $\beta$ -domain found at the C-terminal end of the protein adopts a  $\beta$ -barrel conformation in the OM allowing secretion of the passenger domain into the extracellular space (reviewed in Nishimura et al., 2010). ShdA is widely distributed in *S. enterica* subspecies I and appears to be produced during typhoid fever despite the presence of a frameshifting sequence (Harris et al., 2006; Parkhill et al., 2001). Interestingly, ShdA and MisL can bind fibronectin in other serovars of *Salmonella* and are both considered as pseudogenes in *S. Typhi* (Dorsey et al., 2005; Kingsley et al., 2002). SadA harbours homology to the trimeric autotransporter adhesin YadA of *Yersinia enterocolitica*, a highly repetitive fibrous surface protein (Grosskinsky et al., 2007). YaiU (STY0405) encodes a fourth putative autotransported adhesin with no known role except that antibodies against the protein are produced during a typhoid fever (Harris et al., 2006).

### 3.3 Other adhesins

Besides fimbrial and afimbrial adhesins, other surface-exposed proteins can act as adhesins and mediate crucial roles during typhoid fever. One of the most hydrophobic proteins

encoded in the *S. Typhi* chromosome, STY0351, was recently characterized in detail and might be used as a potential vaccine target. This cell-surface protein is a novel adhesin directly involved in the pathogenesis of *S. Typhi* by conferring strong binding to the laminin extracellular matrix (Ghosh et al., 2011) and is positively regulated by the PhoP-PhoQ two-component system (Charles et al., 2009). It also possesses high immunogenic properties and STY0351-specific antibodies confer protection in a mouse model (Charles et al., 2010; Ghosh et al., 2011). PagC is another surface-exposed protein activated by the PhoP-PhoQ system that is produced and actively recognised by antibodies from patients having previously suffered from typhoid fever (Charles et al., 2010; Harris et al., 2006). Previously associated with survival within macrophages (Miller et al., 1989), PagC possesses serum resistance activity (Nishio et al., 2005) and can promote OM vesicle release in *S. Typhimurium* (Kitagawa et al., 2010), but none of these roles are confirmed yet for *S. Typhi*.

Multivalent adhesion molecules (MAM) are outer membrane proteins harbouring 6 or 7 mammalian cell entry domains and are widely found in pathogenic Gram-negative bacteria (Krachler et al., 2011). MAM mediates early interactions with different cell types by providing protein as well as lipid interactions with fibronectin and phosphatidic acid (Krachler et al., 2011). The specificity for certain cell types is thought to be provided by the other adhesins clustered throughout the genome of the bacteria. In *S. Typhi*, BLASTP analysis revealed that STY1980 harbours about 96% homology with MAM7 of the EPEC strain *E. coli* O127:H6 (Altschul et al., 1990) and could be implicated during the primary interactions with the intestinal mucosa.

#### 4. Capsule and LPS

*S. Typhi* produces a group 1 exopolysaccharide known as the Vi antigen. Thus, *S. Typhi* is one of the few *Salmonella* serovars that get shielded by an extracellular polysaccharide layer constituting the Vi capsule. The Vi polysaccharide is a linear homopolymer of  $\alpha(1\rightarrow4)$ -2-acetamido-3-*O*-acetyl-2-deoxy- $\alpha$ -D-galacturonic acid (Heyns et al., 1959) and constitutes the major component of an injectable conjugated vaccine presently used against typhoid fever world-wide (World Health Organization, 2003). Vi has been involved in pathogenicity by evading the host innate immune system as it protects bacteria from phagocytosis and complement-mediated killing (Kossack et al., 1981). The *in vitro* masking of the OAg by the Vi antigen has been known for a long time (Felix & Pitt, 1934 as cited in Robbins & Robbins, 1984), prevents recognition by TLR-4, and limited C3 deposition to the cell surface (Looney & Steigbigel, 1986), which will lead to reduced clearance of the bacteria (Wilson et al., 2011). Vi is preferentially expressed at low osmolarity and early during infection of human macrophages or mice and will be downregulated with the progression of infection (Daigle et al., 2001; Faucher et al., 2006; Janis et al., 2011). The expression of Vi reduces invasion, probably by limiting the access of the T3SS-1 (Arricau et al., 1998; L. Zhao et al., 2001) or by masking other adhesion molecules including Fim. Vi is also important for surviving in macrophages (Hirose et al., 1997). Vi is tightly regulated by its own activator TviA (Hashimoto et al., 1996; Virlogeux et al., 1996), the two-component system OmpR-EnvZ (Pickard et al., 1994), the Rcs system (Arricau et al., 1998; Virlogeux et al., 1996) and repressed by RpoS (Santander et al., 2007).

Lipopolysaccharide (LPS) is the principal component of the outer membrane of Gram-negative bacteria and a major virulence determinant of many pathogens (Raetz & Whitfield,



2002). It is a glycolipid consisting in three structural regions covalently linked: (i) lipid A, also known as endotoxin, a hydrophobic anchor composed of acyl chains linked to phosphorylated *N*-acetylglucosamine; (ii) the inner and outer core composed of conserved oligosaccharides and; (iii) a variable polysaccharide chain or OAg. *Salmonella* OAg exhibits extensive composition and structural variation and has been divided into 46 O serogroups (Popoff et al., 2001). The O9 antigen of *S. Typhi* is characterized by the presence of a tyvelose residue. In response to acidified macrophage phagosomes, genes activated by the PhoP-PhoQ and PmrA-PmrB systems can modify the global structure of LPS and protect *Salmonella* from being killed by the immune system, notably by antimicrobial peptides (reviewed in Gunn, 2008). Heterogeneity in the length of the OAg repeats has been observed (P. Reeves, 1993) and is important for serum resistance and interaction with host cells (Bravo et al., 2011; Hoare et al., 2006; Hölzer et al., 2009). The *S. Typhi* OAg is essential for serum resistance but is not required for cell invasion (Hoare et al., 2006). Internalization of *S. Typhi* by epithelial cells involves the LPS core (Hoare et al., 2006) which acts as a ligand for CFTR (Lyczak et al., 2001; Pier et al., 1998). The *S. Typhi* LPS core is involved in intracellular replication in macrophages (unpublished data), as observed with *S. Typhimurium* (Nagy et al., 2006; Zenk et al., 2009). *S. Typhi* does not have a bimodal distribution of OAg as it cannot produce very long OAg, consisting of more than 100 repeats of OAg units, because the major regulator Wzz (FepE) is non functional (Raetz & Whitfield, 2002). LPS biosynthesis involves many genes located in different clusters on the chromosome and may be controlled through several regulatory systems (P.R. Reeves et al., 1996). In *S. Typhi*, OAg expression is regulated by RfaH under the control of sigma factor RpoN (Bittner et al., 2002).

## 5. Secretion systems

### 5.1 Type 3 secretion systems

*S. enterica* harbours two distinct type 3 secretion systems (T3SSs) located on SPI-1 (T3SS-1) and SPI-2 (T3SS-2) that are crucial to its virulence along with a flagellar apparatus. T3SSs are complex molecular machines built from more than 20 different proteins, forming a structure similar to a molecular syringe (Kubori et al., 1998, Kimbrough & Miller, 2000 as cited in Sanowar et al., 2010). IM and OM rings are connected by a channel called the needle complex. These structures can inject many protein effectors directly from the bacterial cytoplasm to the cytoplasm of the eukaryotic cells, allowing a direct manipulation of host cellular pathways. The injection process is energized by specific cytoplasmic ATPase and direct contact with the eukaryotic cells is needed in order to activate secretion. Although T3SS are surface-exposed molecules, the lack of specific antibodies against the T3SS in the sera of convalescent patients of typhoid fever (Charles et al., 2010; Harris et al., 2006; Hu et al., 2009) might be a consequence of their tight regulation.

#### 5.1.1 T3SS-1

In order to cause a systemic infection, *Salmonella* must first cross the intestinal epithelial barrier. Conditions found in the intestine, such as low oxygen tension and high osmolarity, are known to induce T3SS-1 of *Salmonella* by the HilA central regulator (Bajaj et al., 1996, Galán & Curtiss, 1990 and Jones & Falkow, 1994 as cited in Altier, 2005). Injection of effectors secreted by the T3SS-1 mediates the invasion of non-phagocytic epithelial cells by *Salmonella* (Galán & Curtiss, 1989; Galán, 1999). Effectors interact with the actin cytoskeleton

and induce membrane ruffles around the bacteria allowing its internalisation into epithelial cells. In *S. Typhi*, the contribution of the T3SS-1 during invasion of epithelial cells was confirmed with *invA*, *sipEBCDA* or *iagAB* (*hilAB*) mutants (Galán & Curtiss, 1991; Hermant et al., 1995; Miras et al., 1995). The T3SS-1 of *S. Typhi* may also play a role during the systemic phase of the infection (Haraga et al., 2008; Libby et al., 2010).

### 5.1.2 T3SS-2

After reaching the epithelial submucosa, *Salmonella* encounters and enters immune system cells like macrophages, dendritic cells and neutrophils. The intracellular environment of these cells promotes induction of the T3SS-2, which is regulated by the SsrA-SsrB two-component regulatory system. Inside cells, bacteria are found in a SCV (*Salmonella*-containing vacuole) and inject T3SS-2 effectors to modify the SCV, alter host pathways and promote intracellular survival (Brumell et al., 2001; Waterman & Holden, 2003; Yu et al., 2004). Although *S. Typhimurium* absolutely requires the T3SS-2 for its intramacrophage survival (Cirillo et al., 1998; Hensel et al., 1998), a complete deletion of this system does not impair survival of *S. Typhi* in human macrophages (Forest et al., 2010). Nevertheless, *S. Typhi* T3SS-2 might be required for survival in other immune cells, as a mutant harbouring a transposon insertion in *ssrB* is disadvantaged in a humanized mouse model (Libby et al., 2010).

### 5.1.3 Flagella

The flagellar apparatus constitutes a third T3SS that is under the control of a highly organized transcriptional hierarchy involving three promoter classes with *flhDC* being the first activator (Kutsukake et al., 1990 and Karlinsey et al., 2000 as cited in Chevance & Hughes, 2008). In *Salmonella*, each cell harbours 6-8 peritrichous flagella built from more than 25 different proteins (Harshey, 2011). The final structure is composed of a basal body, including a stationary and a moving rotor, an external hook and the filament comprised of flagellin (Harshey, 2011). Secretion of flagellin subunits and motility processes are powered by the proton motive force (Minamino & Namba, 2008 and Paul et al., 2008 as cited in Chevance & Hughes, 2008). Subspecies I, II, IIIa and IV of *Salmonella enterica* are considered biphasic since they can alternatively express FliC or FljB major flagellar subunits in a mechanism known as phase variation (Lederberg & Iino, 1956; Simon et al., 1980). Most *S. Typhi* strains do not possess the *fljB* locus and are monophasic, but some isolates contain a 27 kb linear plasmid harbouring the *fljB*:z66 encoding for a novel flagellin (S. Baker et al., 2007; Frankel et al., 1989). Flagella normally contribute to the virulence through motility and chemotaxis (Macnab, 1999), but can also be implicated during biofilm formation (Crawford et al., 2010a). Flagellin can be detected by TLR-5 present at the cell surface of monocytes, dendritic cells and epithelial cells inducing proinflammatory and adaptive immune responses. In *S. Typhi*, TviA directly downregulates flagellar expression thereby avoiding its early recognition by the intestinal mucosa (Winter et al., 2008). Flagellar genes are involved in survival within macrophages or during the systemic phase of infection (Bäumler et al., 1994; Chan et al., 2005; Klumpp & Fuchs, 2007; Libby et al., 2010; Y. Zhao et al., 2002). Nevertheless, the real contribution of the flagellar apparatus is hard to evaluate since expression of the T3SS-1 is co-regulated with the flagella (Eichelberg & Galán, 2000; Saini et al., 2010). Interestingly, patients harbouring antibodies directed against flagella had uncomplicated typhoid fever, while prevalence of anti-OMP (outer membrane proteins) antibodies was associated with increased ileal perforation rates (Nambiar et al., 2009).

5.2 Type 6 secretion systems

Type 6 secretion systems are newly-discovered structures present in about 25% of sequenced Gram-negative bacterial genomes (Boyer et al., 2009). In *S. enterica* subsp. I, T6SS can be identified within SPI-6 (*S. Typhi*), SPI-19, SPI-20 or SPI-21 (Blondel et al., 2009). T6SS are contractile injection machinery harbouring strong similarities to the tail sheath and spike of bacteriophages (Bönemann et al., 2010). These tubular structures can penetrate eukaryotic as well as prokaryotic membranes in a cell-contact dependant way in order to inject protein effectors. T6SS are often required within phagocytic cells (Ma et al., 2009; Pukatzki et al., 2009), but they can also be implicated in biofilm formation (Aschtgen et al., 2008; Enos-Berlage et al., 2005), colonization of the gastrointestinal tract (Blondel et al., 2010), quorum sensing (Weber et al., 2009) as well as in the delivery of toxins to other cells (Hood et al., 2010). Although *S. Typhi* harbours a pseudogene in a key component of its T6SS, the system is functional and its presence corresponds to an enhanced cytotoxicity toward epithelial cells (M. Wang et al., 2011). T6SS expression is regulated by RcsB, PmrA and Hfq (M. Wang et al., 2011). Its contribution during the interaction with hematopoietic cells should be further studied since a transposon insertion in two genes encoded within SPI-6 showed a competitive disadvantage in humanized mice (Libby et al., 2010).

Surface structure	Role in virulence	Observed for <i>S. Typhi</i>	Observed in other serovars
Bcf and Stb	Seroconversion Intestinal persistence in mice	Harris 2006; Hu 2009	Weening 2005
Fim	Binds to mannose, adhesion and invasion of epithelial cells Biofilm	Satta 1993	Althouse 2003 Boddicker 2002
Stg	Adhesion to epithelial cells	Forest 2007	
Sth	Long-term infection of mice		Lawley 2006
Saf	Intestinal colonization of swine Long-term infection of mice		Carnell 2007 Lawley 2006
Sef	Interaction with macrophages		Edwards 2000
Stc	Intestinal persistence in mice		Weening 2005
Std	Binds to $\alpha(1,2)$ fucose Intestinal persistence in mice		Chessa 2009 Weening 2005
Tcf and YaiU	Seroconversion	Harris 2006	
Csg	Seroconversion Biofilm	Harris 2006	Ledeboer 2006
SiiE	Adhesion to apical side of epithelial cells Colonization of the gastrointestinal tract		Gerlach 2008 Blondel 2010
BapA	Interaction with intestinal mucosa, bacterial autoaggregation and biofilm		Latasa 2005
ShdA	Seroconversion Binds to fibronectin	Harris 2006	Kingsley 2002
MisL	Intestinal colonization, binds to fibronectin		Dorsey 2005

Surface structure	Role in virulence	Observed for <i>S. Typhi</i>	Observed in other serovars
Type IVB pili	Binds to CFTR, cellular invasion Bacterial self-association	Pan 2005; Pier 1998 Morris 2003b	Morris 2003a
STY0351	Seroconversion Cell adhesion and binds to laminin	Charles 2010 Ghosh 2011	
PagC	Seroconversion Survival within macrophages Serum resistance OM vesicle release	Charles 2010; Harris 2006	Miller 1989 Nishio 2005 Kitagawa 2010
Vi Capsule	Host immune system evasion Intramacrophage survival	Kossack 1981; Looney, 1986; Wilson 2011 Hirose 1997	
LPS	Binds to CFTR Antimicrobial peptides resistance Serum resistance Intramacrophage survival	Lyczak 2001 Baker 1999 Hoare 2006 Unpublished data	Gunn 2008 Bravo 2008 Nagy 2006
T3SS-1	Effectors secretion and invasion of eukaryotic cells	Galán 1991; Hermant 1995	Galán 1989, 1999
T3SS-2	Effectors secretion and intramacrophage survival		Cirillo 1998; Hensel 1998
Flagella	Motility and chemotaxis Intramacrophage survival Biofilm formation	Liu 1988 Unpublished data	Macnab 1999 Bäumler 1994 Crawford 2010a
T6SS	Colonization of the gastrointestinal tract		Blondel 2010

Table 1. *S. Typhi* surface structures considered in this review and their roles in virulence.

6. Future perspectives

The multidrug-resistance observed for *S. Typhi* strains is of great concern since the total number of cases has increased during the last decade (Crump et al., 2004; Pang et al., 1998). There are two crucial lines of defence that should be improved in order to win the combat against typhoid fever : prevention and treatment. The best vaccine would be safe, given in a single dose, offering an efficient and long lasting immunity and remain stable at room temperature. Next generation vaccines have been recently tested in human trials (reviewed in Lindow et al., 2011). The expression of surface structures is tightly coordinated to avoid recognition by the immune system. Nevertheless, we have some clues regarding the structures recognized during typhoid fever (Charles et al., 2010; Harris et al., 2006; Hu et al., 2009). Since antibodies promote killing of *S. Typhi* (Lindow et al., 2011), a good approach to improve the efficiency of vaccines might be to create an avirulent strain expressing its immunogenic structures on inducible promoters inside antigen presenting cells (S. Wang et al., 2011).

Another strategy in the fight against *S. Typhi* should be the identification and treatment of the 1-5% infected individuals who become asymptomatic carriers (Crawford et al., 2010b; Parry et al., 2002). This task is complicated as antibiotherapy is often unsuccessful to remove



biofilms found in the gallbladder, especially on gallstones, and surgical removal of the gallbladder is usually required but expensive (Crawford et al., 2010b; Prouty et al., 2002). Hence, efforts should be taken to understand the specific structures required for biofilm formation by *S. Typhi* in order to develop therapies to eliminate typhoid carriage.

Novel strategies are being developed to target surface structures implicated in bacterial pathogenesis as potential treatments (reviewed in Lynch & Wiener-Kronish, 2008). For example, pilicides are small compounds preventing interactions between the OM usher and chaperone-subunits complexes of type 1 pili, hence interfering with fimbrial biogenesis (Pinkner et al., 2006). Since most surface structures of *Salmonella* are expressed by the CUP, targeting the fimbrial ushers might be a useful method to eliminate colonisation and avoid the resulting antimicrobial resistance. Moreover, curlicides are able to interfere with CsgA polymerization as well as type 1 fimbrial biogenesis resulting in the blocking of biofilm accumulation (Cegelski et al., 2009). Similarly, small-molecule inhibitors and inactivating antibodies can target binding or translocation of effectors by T3SS (Hudson et al., 2007; Neely et al., 2005; Nordfelth et al., 2005; Swietnicki et al., 2011). Targeting the capsule or LPS biosynthetic pathways might be a good approach to fight against *S. Typhi* since there is no corresponding enzyme in its human host (Cipolla et al., 2010; Goller & Seed, 2010).

Finally, understanding the role and function of *S. Typhi* surface proteins is primordial as these molecules are the first ones to directly interact with host components or cells, leading to a possibility for the development of new strategies to fight typhoid (see Table 1).

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