UNIVERSIDAD Ð SEVILLA

Genetic and molecular analysis of the *std* operon in *Salmonella enterica*

Lucía García Pastor

Marzo 2017

Genetic and molecular analysis of the *std* operon in *Salmonella enterica*

Memoria elaborada por Lucía García Pastor para optar al grado de Doctor en Biología

Tesis realizada bajo la dirección del Dr. Josep Casadesús Pursals y la Dr. Elena Puerta Fernández en el Departamento de Genética de la Facultad de Biología de la Universidad de Sevilla

Josep Casadesús Pursals

Elena Puerta Fernández

Lucía García Pastor

Sevilla, Marzo de 2017

A mi familia, a Guille
y a los BACT

“Ve hasta donde te alcance la vista. Cuando
llegues, serás capaz de ver más allá”.

TOMAS CARLYLE

INDEX

RESUMEN

Salmonella enterica serovar Typhimurium es una bacteria gram-negativa que causa enfermedades gástricas y sistémicas en una gran variedad de organismos, incluyendo la especie humana. La invasión del epitelio intestinal es un proceso crítico en la infección por *Salmonella* y requiere funciones codificadas en una región del cromosoma bacteriano adquirida por transferencia horizontal, conocida como isla de patogenicidad 1 (SPI-1). Otro *locus* que *Salmonella* ha adquirido por transferencia horizontal es el operón *std*, implicado en la formación de fimbrias que participan en la colonización del intestino del hospedador.

La expresión del operón *std* se encuentra reprimida en condiciones de laboratorio y se activa en el intestino. Estudios previos han mostrado que esta represión es causada principalmente por la metilación Dam y en menor medida por el represor RosE. La transcripción del operón *std* en los mutantes Dam$^-$ requiere HdfR, un factor de transcripción poco conocido de tipo LysR. Estos mismos estudios han sugerido la posibilidad de que la expresión de *std* esté sujeta a biestabilidad o cambio de fase. Recientemente se ha visto que el producto de los dos genes distales del operón, *stdE* y *stdF*, es responsable de la represión de los genes de SPI-1 en mutantes Dam$^-$, que muestran un fenotipo de baja invasión en células epiteliales.

En base a estos antecedentes, esta Tesis se inició con el objetivo de investigar si la expresión del operón *std* está sujeta a biestabilidad en condiciones de laboratorio. La existencia de heterogeneidad fenotípica en poblaciones clonales es un concepto relativamente reciente, pero cada vez son más los ejemplos encontrados. Para determinar si el operón *std* mostraba biestabilidad se construyeron fusiones GFP en *stdA* y se usó citometría de flujo para analizar su expresión en células individuales. De estos experimentos se ha podido concluir que existe una expresión bimodal del operón *std* en condiciones de laboratorio. La expresión de *std* tiene lugar en un 0,3-3% de la población, generando dos subpoblaciones: Std^{ON} y Std^{OFF}.

Otro objetivo de esta Tesis era entender el mecanismo molecular que controla la transcripción de *std* generando una expresión bimodal del mismo. Se ha puesto de manifiesto el papel esencial de HdfR para la expresión de *std*, no solo en fondo Dam$^-$ como ya se había descrito sino también en fondo silvestre. La subpoblación Std^{ON} desaparece en ausencia de HdfR y aumenta de tamaño si *hdfR* se expresa constitutivamente. Además, igual que ocurre en otros factores transcripcionales de tipo LysR, hemos demostrado la existencia de un proceso de autorregulación (represión) que mantiene la transcripción de *hdfR* en niveles constantes.

La metilación Dam es el principal represor de la expresión del operón *std*, pero existen otros represores previamente descritos como RosE o SeqA. En colaboración con el grupo de la Dr. Delgado del Instituto Superior de Investigaciones Biológicas (INSIBIO) CONICET-UNT en Argentina, se ha identificado un nuevo represor de la expresión de *std*, RcsB. La expresión constitutiva de *rcsB* reprime la expresión de *std* en fondo Dam^- y también en fondo silvestre. Se han realizado ensayos de retardo en gel para corroborar la unión de RcsB a la región promotora de *std*. El análisis bioinformático identifica un posible sitio de unión de RcsB en la región -35 del promotor de *std*.

Con el fin de identificar otros posibles reguladores de la transcripción del operón *std*, se realizaron escrutinios genéticos. Los resultados obtenidos mostraron una autorregulación positiva del operón *std*, de la que son responsables los genes distales del operón *std*: *stdE* y *stdF*. Ensayos realizados mediante citometría de flujo corroboran los resultados del escrutinio. La expresión de *stdE* y *stdF* mantiene la subpoblación Std^{ON} y una expresión constitutiva de *stdE* y *stdF* aumenta el tamaño de dicha subpoblación.

La expresión bimodal del operón *std* en fondo silvestre nos llevó a pensar en la existencia de patrones de metilación distintos para cada una de las subpoblaciones. Para demostrar la existencia de dichos patrones de metilación se han realizados ensayos de Southern Blot tras digestión del DNA con isosquizómeros de enzimas de restricción que reconocen la misma secuencia en el DNA (GATC) pero difieren en su sensibilidad al estado de metilación de la diana. Los tres sitios GATC de la región reguladora se hallan metilados cuando el promotor está inactivo (la subpoblación Std^{OFF} es mayoritaria en un fondo silvestre). Resultados de citometría de flujo sugieren un patrón de metilación distinto para la subpoblación Std^{ON}, ya que la hipermetilación disminuye dicha subpoblación.

Anteriormente se había descrito que StdE y StdF controlan la expresión de *hilD* a nivel postranscripcional. Teniendo en cuenta que nuestros resultados indican que StdE y StdF son reguladores autógenos de la transcripción de *std*, nos planteamos la posibilidad de que estas proteínas tuvieran un papel regulador. Con objeto de identificar posibles dianas de regulación del regulón StdEF se realizó un análisis transcriptómico usando un *microarray* de *S. enterica* serovar Typhimurium. Los datos obtenidos nos han permitido identificar a StdE y StdF como reguladores globales de la transcripción en *Salmonella*, ya que no sólo reprimen la isla de patogenicidad 1 (SPI-1) sino también genes flagelares y de quimiotaxis. Por otra parte, StdE

y StdF activan la expresión de genes del plásmido de virulencia y del gen *hdfR*, que codifica el factor de transcripción esencial para la expresión del operón *std*.

Para tratar de determinar el mecanismo regulador de estas proteínas, hemos realizado un ensayo de ChIP-seq (inmunoprecipitación de cromatina seguida de secuenciación masiva), que permite identificar sitios de unión al DNA de las proteínas analizadas. Los resultados obtenidos confirman el papel de StdE y StdF como reguladores transcripcionales de la expresión en *Salmonella*. StdE parece tener un papel principal, uniéndose a regiones promotoras del DNA. Por otro lado, los resultados del ChIP-seq sugieren un papel secundario o accesorio de StdF en la regulación, quizá favoreciendo la actuación de StdE. El análisis global de la unión de StdE y StdF al genoma de *Salmonella* ha proporcionado información acerca de la autorregulación del operón *std*. Como ya se ha mencionado, StdE y StdF favorecen la expresión de *std*, aumentando el tamaño de la subpoblación Std^{ON}. Según los datos del ChIP-seq, es StdF quien se encuentra en la región promotora, favoreciendo la expresión de *std*. Queda por determinar si StdF tiene un papel principal en la regulación de *std* o, como en otros sitios de regulación, actúa de modo auxiliar, tal vez favoreciendo la unión de HdfR o impidiendo la actividad de la metilasa Dam.

Todo parece indicar que nos encontramos frente a un sistema de regulación global pero con expresión bimodal. La expresión del operón *std*, con la consiguiente formación de fimbrias, tiene lugar en una pequeña fracción de la población en condiciones de laboratorio, y mayoritariamente en condiciones de adherencia al epitelio intestinal. En dichas condiciones, la bacteria mantiene reprimidos los genes implicados en virulencia, expresión del flagelo o quimiotaxis, y los genes reguladores del operón *std* son los encargados de coordinar estos procesos. Por otro lado la expresión del operón *std* favorece la activación del sistema conjugativo del plásmido de virulencia y garantiza el mantenimiento del mecanismo autorregulador de expresión del propio operón *std* al activar el factor de transcripción HdfR y al ejercer de reguladores transcripcionales uniéndose a la región promotora del propio operón *std*.

INTRODUCTION

The genus *Salmonella*

The genus *Salmonella* includes facultative anaerobic Gram-negative, rod-shaped bacteria that are able to infect a variety of animal hosts including amphibians, reptiles, birds and mammals. Salmonellae are usually motile and produce peritrichous flagella. *Salmonella* belongs to Enterobacteriaceae family in the γ-proteobacteria subdivision and is a close relative of the genera *Escherichia*, *Shigella* and *Citrobacter*.

Currently, the genus *Salmonella* is divided into 2 species, known as *Salmonella enterica* and *Salmonella bongori* (Tindall et al., 2005). *Salmonella enterica* comprises 6 subspecies (McQuiston et al., 2008): *enterica* (subsp. I), *salamae* (subsp. II), *arizonae* (subsp. IIIa), *diarizonae* (subsp. IIIb), *houtenae* (subsp. IV) and *indica* (subsp. VI).

Salmonella subspecies are further divided into serovars (also known as serotypes). Serovars are defined by the antigen properties of the polysaccharide chain of LPS (O-antigens) and of the proteinaceous flagella (H antigens) (Grimont & Weill, 2007; McQuiston et al., 2004). There are more than 2,500 *Salmonella* serovars, most of them belong to the subsp. *enterica* (Popoff et al., 2004). Only serovars of this subspecies are able to colonize warm-blooded vertebrates (McClelland et al., 2001), accounting for 99% of human infections caused by *Salmonella*, while the remaining subspecies of *Salmonella enterica* and members of *Salmonella bongori* are usually associated to cold-blooded vertebrates or to the environment (Bäumler et al., 1998).

Differences in host specificity and types of diseases produced are found among serovars belonging to subsp. *enterica.* Some serovars are able to infect a wide variety of animals, while others are host-specific (Bäumler & Fang, 2013). Serovars of subsp. *enterica* produce a variety of diseases that ranges from self-limiting gastroenteritis to life-threatening systemic infection, and the outcome of the disease depends on the specific serovar-host combination. For example, the human-restricted serovar Typhi produces typhoid fever, while the generalist serovar Typhimurium produces mild gastroenteritis in humans but causes a systemic infection similar to human typhoid fever when infecting mice (Velge et al., 2012). For this reason, the interaction between serovar Typhimurium and mice has been widely used as a model for human typhoid fever (Santos et al., 2001a), and most pathogenicity studies in *Salmonella* have employed this serovar. In this work we have used the mouse-virulent strain

Salmonella enterica subsp. *enterica* serovar Typhimurium SL1344, from the B.A.D. Stocker collection. For simplicity, it will be often abbreviated as *Salmonella typhimurium*.

Evolution of *Salmonella* pathogenesis

Salmonella is a close relative of *Escherichia*. They diverged 120-160 million years ago (Ochman & Wilson, 1987). Almost 25% of the genetic material in the *Salmonella* genome is absent in *Escherichia coli* (McClelland et al., 2001; Porwollik & McClelland, 2003). The evolution of *Salmonella* pathogenicity **(Figure I. 1)** has involved the sequential acquisition of genetic elements, each contributing to different aspects of its lifestyle (Groisman & Ochman, 1997; Kelly et al., 2009). Among those elements are the *Salmonella* pathogenicity islands (SPIs), which are clusters of virulence genes in the chromosome. More than 10 SPIs have been described (Hensel, 2004) including some that are serotype-specific. These regions usually have a different G+C content than the rest of the *Salmonella* chromosome, and are absent in other Enterobacteriaceae, suggesting that they have been acquired by horizontal gene transfer (Kelly et al., 2009; Porwollik & McClelland, 2003).

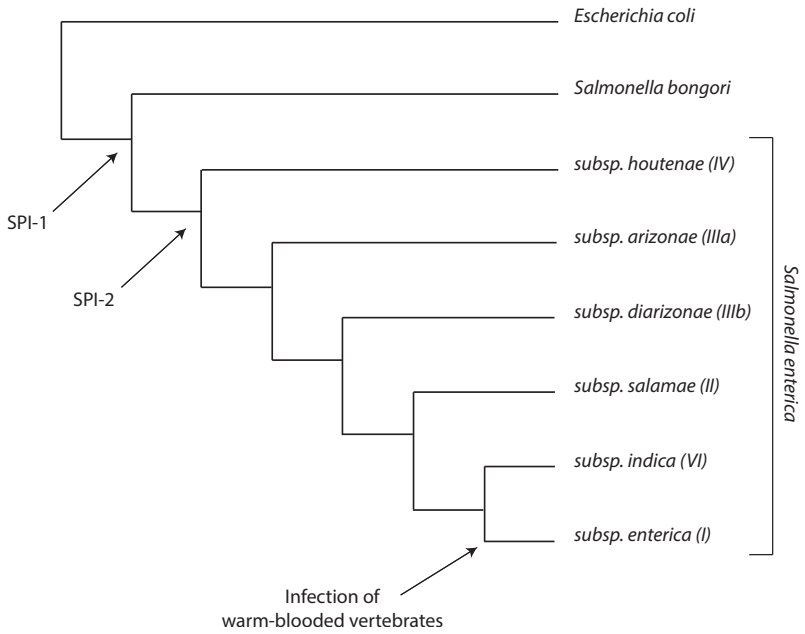

Figure I. 1 Phylogeny of the genus *Salmonella*. The acquisition of SPI-1 and SPI-2, and the ability to infect warm-blooded vertebrates are indicated. Modified from (Ellermeier & Slauch, 2006).

Salmonella pathogenicity island 1 (SPI-1) and *Salmonella* pathogenicity island 2 (SPI-2) are the best characterized SPIs. SPI-1 was first acquired by the common ancestor of *Salmonella bongori* and *Salmonella enterica*, and is involved in the invasion of epithelial cells in the animal intestine (Que et al., 2013). SPI-1 acquisition provided *Salmonella* the ability to invade eukaryotic cells and to become an intracellular pathogen, likely associated with cold-blooded vertebrates (Ellermeier & Slauch, 2006). SPI-2 allows *Salmonella* to survive in macrophages and colonize deeper tissues (Ochman et al., 1996), and its acquisition resulted in the split of the two *Salmonella* species (Ellermeier & Slauch, 2006). Consequently, only members of *Salmonella enterica* are able to reach deep tissues and organs producing systemic infection.

The ancestor of subsp. *enterica* acquired the capacity to infect warm-blooded vertebrates, and different strains subsequently evolved to colonize a variety of hosts. Although the mechanism of host specificity is not fully understood, the presence of a virulence plasmid in certain serovars of subsp. *enterica* has suggested the potential contribution of plasmid functions (Bäumler et al., 1998). Another trait that may be involved in host specificity is the presence of different sets of fimbrial operons in different serovars (Bäumler et al., 1998; Townsend et al., 2001).

Salmonella infection

Salmonella enterica is found mostly in the intestine of animal hosts and the infection is usually transmitted by the fecal-oral route. Infection normally starts via ingestion of contaminated water or food. *Salmonella* must endure adverse conditions along the digestive track, which serves as a protective barrier against bacterial infections.

The acid pH in the stomach destroys the majority of microorganisms (Tennant et al., 2008). However, *Salmonella* responds with the activation of the acid tolerance response, a complex adaptive system, which allows the bacterial population to endure periods of severe acid stress (Foster & Hall, 1990; Lee et al., 1995).

High concentrations of bile are secreted in the small intestine, in particular in the duodenum, during digestion. Bile has two main antibacterial actions: as a detergent disrupting the cell envelope (Gunn, 2000) and as DNA damaging agent producing DNA rearrangements and

point mutations (Prieto et al., 2004). Nevertheless, like other enteric bacteria, *Salmonella* is intrinsically resistant to high concentrations of bile (Gunn, 2000).

Once in the distal small intestine, *Salmonella* has the ability to penetrate inside epithelial cells. In a first step, adhesins and fimbriae are necessary to mediate the adherence (Wagner & Hensel, 2011); afterwards the bacterial population is able to invade the intestinal epithelium through three different routes (**Figure I. 2**): (i) by inducing a phagocytosis-like process in non-phagocytic enterocytes, (ii) through specialized epithelial M cells, and (iii) through dendritic cells that intercalate epithelial cells by extending protrusions into the gut lumen (Finlay & Brumell, 2000; Grassl & Finlay, 2008). The two first routes are mediated by the virulence-associated type III secretion system encoded on *Salmonella* pathogenicity island 1 (SPI-1) (Zhou & Galán, 2001), with invasion of M cells being the predominant route of intestinal traversal (Watson & Holden, 2010).

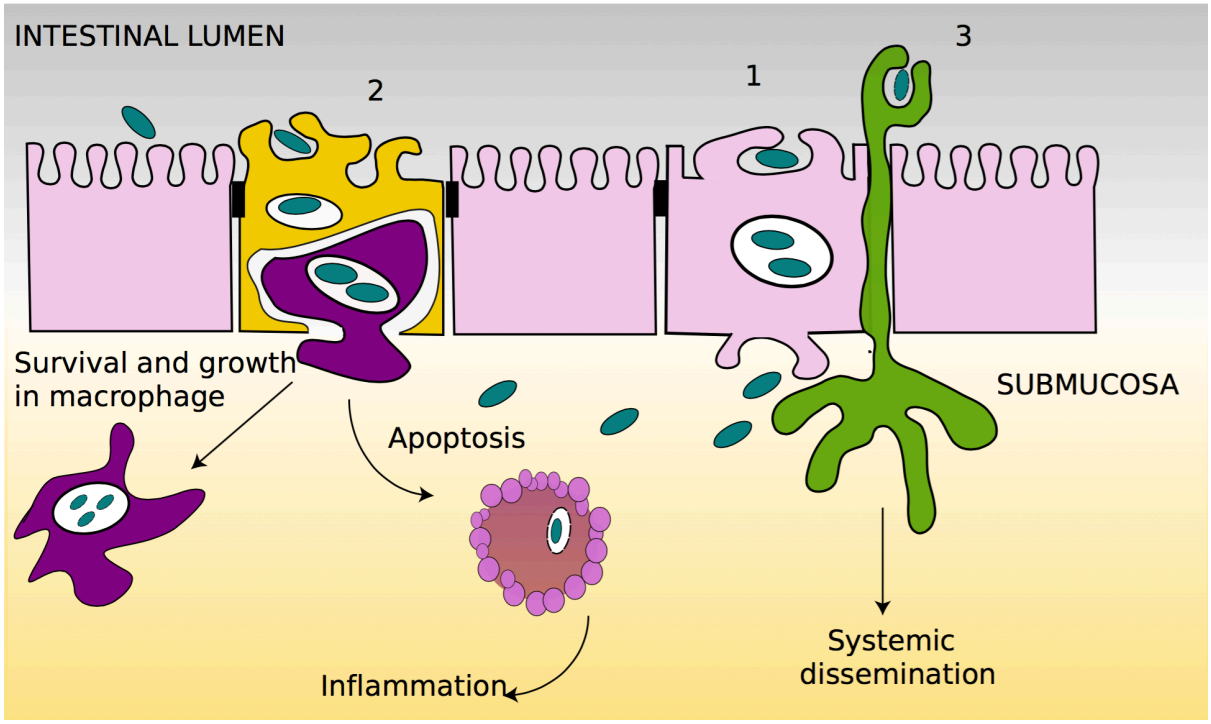

Figure I. 2 Diagram of *Salmonella* infection. The three main routes of *Salmonella* invasion of the intestinal epithelium are represented: direct invasion of intestinal epithelial cells (1), adhesion and translocation through M cells (2), and capture by dendritic cells (3). Adapted from (Fàbrega & Vila, 2013; Sansonetti, 2004).

Depending on the serovar and the host, *Salmonella* infections may have three different outcomes after invasion of intestinal epithelial cells: gastroenteritis, systemic infection, or asymptomatic chronic carriage.

In gastroenteritis, the infection is localized in the intestine. Invasion of intestinal epithelial cells triggers an inflammatory reaction in the intestinal mucosa. Liquid accumulation in the intestinal lumen leads to diarrhea (Nunes et al., 2010; Santos et al., 2001b).

In systemic infection, the pathogen crosses the epithelial barrier and is able to survive inside phagocytes due to functions encoded in SPI-2. *Salmonella* disseminates through the lymphatic system reaching deep tissues and colonizing target organs, particularly the spleen, the liver, the gall bladder and the bone marrow, where bacteria can proliferate, eventually causing death upon septic shock (Bäumler et al., 2011).

A fraction of individuals that has survived systemic infection becomes asymptomatic, life-long carriers of *Salmonella*, acting as reservoirs for future infections. In humans, serovar Typhi can undergo chronic carriage in the gall bladder (Bäumler et al., 2011).

Salmonella virulence factors

The interaction between *Salmonella* and the host requires a complex bacterial machinery to colonize and succeed in a variety of host environments, all of them challenging. This machinery includes genes required for chemotaxis, motility, adhesion, invasion, replication and survival within host cells covering the whole pathogenic process from the intestinal step to systemic dissemination. The virulence factors employed by the pathogen to achieve the infection process are described below.

Type III secretion systems (TTSSs)

Type III secretion systems (TTSSs) are specialized organelles whose main role is to inject bacterial proteins, called effectors, into eukaryotic cells (Cornelis & Van Gijsegem, 2000; Galán, 1999; Galán & Collmer, 1999), representing the principal virulence factors for *Salmonella* pathogenicity (LaRock et al., 2015). TTSSs are evolutionarily related to the flagellar export apparatus and are present not only in bacteria pathogenic for animals and plants but also in symbionts for plants or insects (Galán, 2001).

TTSSs are usually composed of more than 20 proteins (Galán, 2001) that form a needle-like structure that spans both the inner and outer membranes of the bacterial envelope (Kubori et al., 1998). It is composed of a basal body localised in the bacterial membrane, a needle that protrudes outside the cell, and a translocon that can cross the eukaryotic plasmatic membrane (Moest & Méresse, 2013).

Salmonella enterica encodes two TTSSs, and the corresponding genes are located in pathogenicity islands (SPI-1 and SPI-2). Each TTSS exerts its function at a different stage of the infection process. *Salmonella* pathogenicity island 1 (SPI-1) is located at centisome 63 of the chromosome (Galán, 1999) and is required for the initial interaction of *Salmonella* with intestinal epithelial cells (Galán, 2001). *Salmonella* pathogenicity island 2 (SPI-2) is located at centisome 31 and it is required for survival within macrophages during systemic infection (Hensel, 2000).

Regulation of *Salmonella* pathogenicity island 1 (SPI-1)

SPI-1 is a 40 Kb region of the *Salmonella* chromosome that contains all the genes necessary to produce a functional TTSS, several secreted effectors, and transcriptional regulators of bacterial invasion (Ellermeier & Slauch, 2007). The control of invasion involves regulators encoded both inside and outside SPI-1 that are able to integrate environmental signals and molecular factors (Bajaj et al., 1996).

SPI-1 is tightly regulated by four transcriptional activators encoded within SPI-1 (HilA, HilC, HilD, and InvF) and by another regulators encoded outside the island such as RtsA or HilE. HilA belongs to the OmpR/ToxR family and hast raditionally been considered the master regulator of SPI-1, playing a central role in invasion (Bajaj et al., 1995). In support of this view, deletion of *hilA* is phenotypically equivalent to a deletion of the entire SPI-1 locus (Ellermeier et al., 2005). HilA controls genes encoding all the components necessary to build a functional TTSS (Bajaj et al., 1995; Lostroh & Lee, 2001) and indirectly regulates effector proteins by activating *invF* transcription (Bajaj et al., 1995; 1996; Darwin & Miller, 1999). Expression of *hilA* is controlled by the AraC-like activators HilD, HilC, and RtsA (Ellermeier & Slauch, 2003; Schechter & Lee, 2001). These regulators act by binding to the *hilA* promoter, as well as by inducing their own expression or activating other regulators (Boddicker et al., 2003; Ellermeier et al., 2005).

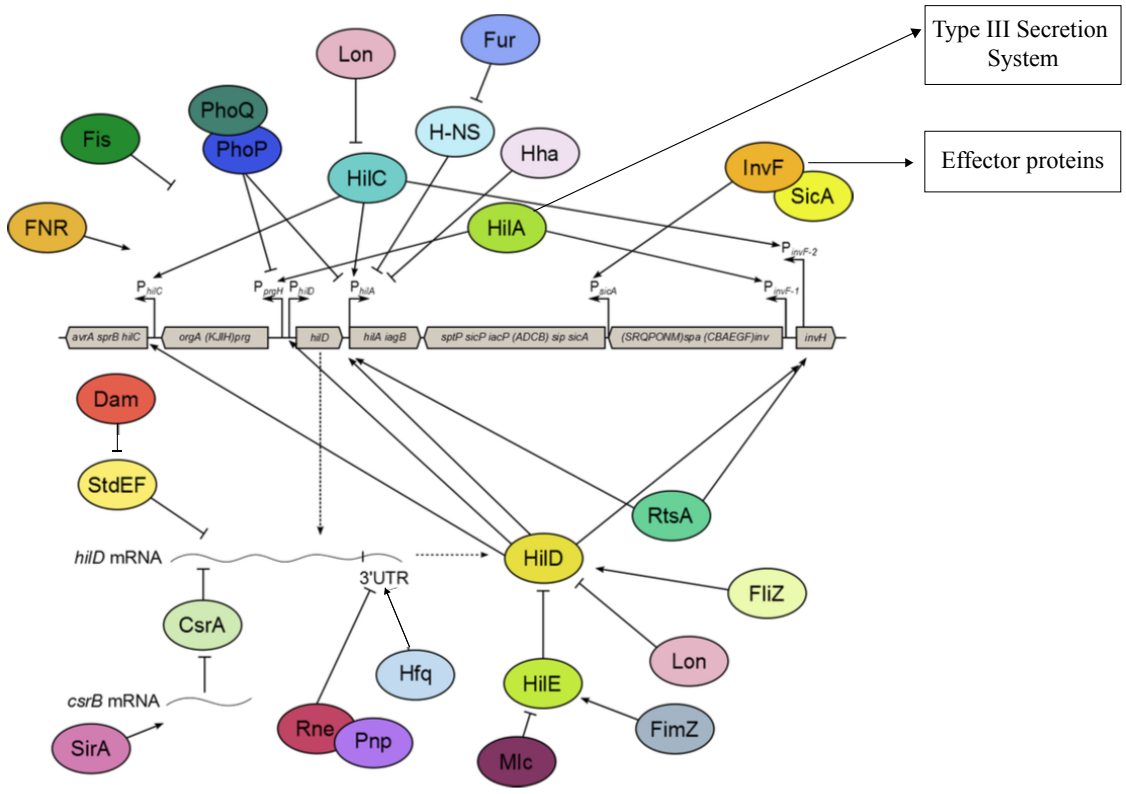

Figure I. 3 Diagram of the regulatory network of SPI-1. Lines ending in arrowheads indicate positive effects, whereas blunt lines indicate negative regulatory effects. Adapted from (Espinosa Alfaro, 2015).

Recent studies describe HilD as one of the most relevant regulators of SPI-1, regulating SPI-1 expression at different levels. HilD is able to regulate the expression not only of *hilA* but also of *hilC, invF and rtsA* upon binding upstream their promoters (Akbar et al., 2003; Olekhnovich & Kadner, 2002; Schechter & Lee, 2001). Various environmental signals, sensed by these regulatory systems, are integrated at the level of HilD production. Together with HilC and RtsA, HilD is involved in a complex feed-forward regulatory loop that can amplify the activation signal and act as a switch for *hilA* transcription (Ellermeier & Slauch, 2007). The synthesis of HilD is subjected to tight regulation at the transcriptional and postranscriptional levels. RtsA, HilC, and HilD itself mediate *hilD* transcriptional regulation by binding to the *hilD* promoter (Ellermeier et al., 2005; Olekhnovich & Kadner, 2002). SirA and StdEF regulate HilD at the postrancripitonal level (López-Garrido & Casadesús, 2012; Teplitski et al., 2003). Recently, it was shown that the long 3'UTR of *hilD* mRNA mediates its decay, and it has been described as a target for the Hfq chaperone and for the *Salmonella* degradosome (López-Garrido et al., 2014). FliZ positively regulates SPI-1 at the level of HilD protein. HilE, a negative SPI-1 regulator, interacts with HilD interfering with its function (Baxter et al., 2003). HilD activity is not limited to SPI-1 regulation. Crosstalk

between SPI-1 and SPI-2 through HilD has been previously reported (Bustamante et al., 2008; la Cruz et al., 2015). HilD also activates transcription of the flagellar master operon *flhDC*, which is essential for *Salmonella* motility (Singer et al., 2014). Recently, novel targets for HilD have been described, many of them outside SPI-1 (Petrone et al., 2014; Smith et al., 2016).

Flagellum and chemotaxis

The bacterial flagellum permits bacterial motility in liquid environments. The general structure and organization of the flagellum are well conserved among Gram-negative bacterial species. The flagellum constitutes a supramolecular complex formed by at least three parts: the basal body (reversible motor), which is joined to the filament (helical propeller) by the hook (universal joint). Four rings and a rod compose the basal body. The rings C, MS, P, and L are located in the cytoplasm, cytoplasmic membrane, peptidoglycan layer and outer membrane respectively. The rod is a drive shaft that traverses the periplasmic space. In response to chemotactic signals, the flagellar motor can operate in both the counter-clockwise and clockwise directions, switching the direction of motor rotation (Minamino & Imada, 2015).

The assembly and function of the flagellar and chemotaxis systems require the expression of more than 50 genes, divided in operons (Macnab, 2003) that are regulated at several levels in response to environmental and flagellar development signals (Chilcott & Hughes, 2000). In *Salmonella*, the flagellar, motility and chemotaxis genes form a regulon, and they are classified into three temporally regulated transcription classes: early, middle and late. (Kutsukake et al., 1990). The corresponding promoters are referred to as class 1, class 2 and class 3, respectively. Class 1 includes a single promoter that transcribes the *flhDC* operon. FlhD and FlhC are active as an heterotetramer which is a key transcriptional activator of middle class promoters. This transcription responds to many environmental cues. Expression of middle class genes is necessary for the synthesis and assembly of the hook-basal body, and the transcriptional regulators FlgM and σ^{28} (*fliA*) are necessary for expression of late genes (Chilcott & Hughes, 2000). Late genes encode proteins later required in the assembly process, including flagellin, hook-associated proteins, stator components and chemosensory systems. Expression of late genes is a critical step for flagella production. FliA and FlgM

regulate, positively and negatively, class 3 promoters respectively (Gillen & Hughes, 1991; Kutsukake & Iino, 1994).

Reduced motility has been observed in Dam⁻ mutants in *E. coli* (Oshima et al., 2002) and *Salmonella* (Balbontín et al., 2006). Despite the increased expression of some flagellar genes like *fliC* or *fliD* in Dam⁻ strains, reduced halo formation on motility plates is observed in Dam⁻ mutant background (Balbontín et al., 2006).

Flagellar and motility genes are expressed at the early exponential growth phase, and their expression is subject to crosstalk with other *Salmonella* virulence determinants. For example, the flagellar activator FliZ (middle gene class) has been reported to increase *hilA* transcription in a HilD-dependent manner, suggesting that FliZ activates *hilA* at postranscriptional level controlling HilD (Chubiz et al., 2010) and simultaneously repressing fimbrial genes (Saini et al., 2010).

The *Salmonella enterica* virulence plasmid

In *Salmonella* and in other bacteria such as *E. coli, Shigella* or *Yersinia*, certain virulence factors important for pathogenesis are encoded on plasmids. In *Salmonella,* plasmids have been found in a few serovars belonging to the subspecies I. These plasmids, called "serovar-specific" have sizes of 50 to 90 Kb. They are very stable and their copy number is usually 1 or 2 copies per cell (Rotger & Casadesús, 1999).

The virulence plasmid in *S.* Typhimurium, pSLT (Jones et al., 1982), was shown to be mobilizable (Ahmer et al., 1999; García-Quintanilla & Casadesús, 2011; Jones et al., 1982; Ou et al., 1990; Sanderson et al., 1983) and self-transmissible by conjugation (Ahmer et al., 1999).

Expression of the transfer region (*tra*), encoded in the pSLT (**Figure I. 4**), permits pilus biogenesis, mating pair stabilization, DNA transfer, and surface exclusion. During the conjugation process, a donor cell build a conjugative pilus that recognizes a recipient cell, retracts, and brings the two cells to close contact. Plasmid DNA is then transferred to the recipient cell through the mating pore, converted to double-strand DNA, re-circularized, and established in the recipient cell. pSLT transfer increases in the ileum, the distal portion of the small intestine (García-Quintanilla et al., 2008), which may compensate plasmid loss during

intestinal passage (García-Quintanilla et al., 2006). The virulence plasmid also contains the *pef* (plasmid-encoded fimbriae) locus, which is involved in bacterial adhesion to intestinal epithelial cells (Bäumler et al., 1996). The *spv* (*Salmonella* plasmid virulence) region, required for the systemic phase of disease (Gulig et al., 1993), is also encoded in the pSLT plasmid, among others loci (**Figure I. 4A**)

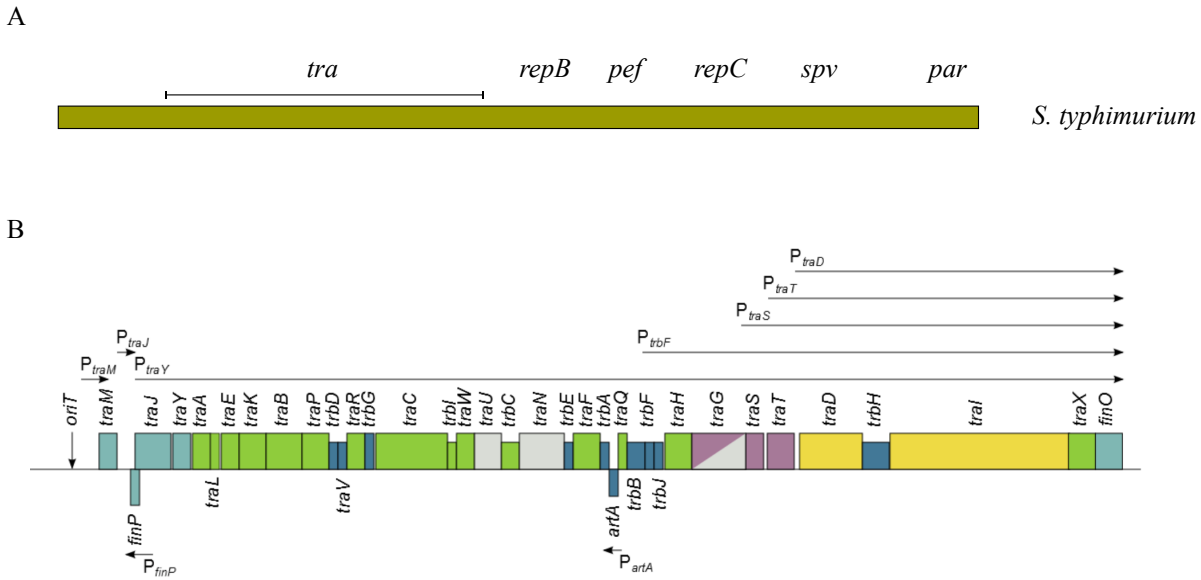

Figure I. 4 pSLT genetic map in *Salmonella enterica.* A. Linear genetic map of the pSLT virulence plasmid of *Salmonella enterica* serovar Typhimurium. **B**. *tra* region in pSLT plasmid in *S. enterica*. Arrows represent transcripts initiated at the highlighted promoter. Adapted from (Bäumler et al., 1998; Espinosa Alfaro, 2015).

Regulation of *tra* operon expression is complex and involves regulatory proteins encoded on both the host chromosome and the plasmid. Host-encoded factors involved in mating and pili synthesis include global regulators such as ArcA (Silverman et al., 1991), H-NS (Starcic-Erjavec et al., 2003; Will et al., 2004), Lrp (Camacho & Casadesús, 2002), or Dam methylation. Dam methylation represses *tra* operon expression by controlling Lrp-mediated activation of *traJ* transcription (Camacho & Josep Casadesús 2002) and by maintaining high levels of FinP RNA (Torreblanca et al., 1999), among others.

FinOP fertility inhibition system controls conjugation at the postranscriptional level. Synthesis of TraJ is controlled by FinP, an antisense RNA that inhibits *traJ* mRNA translation (Firth et al., 1996; Zatyka & Thomas, 1998). FinP binds to *traJ* mRNA sequestering its ribosomal binding site (RBS) and preventing its translation (Koraimann et al., 1996). The duplex FinP/*traJ* mRNA formed is then rapidly degraded by the RNase III.

FinO binds FinP preventing its degradation by RNase E (Jerome et al., 1999) and allowing the FinP concentration to increase to sufficient levels to mediate *traJ* repression.

On the other hand, TraM is essential for nicking and unwinding the plasmid DNA during conjugation . In additon, TraM binds its own promoter and represses its transcription.

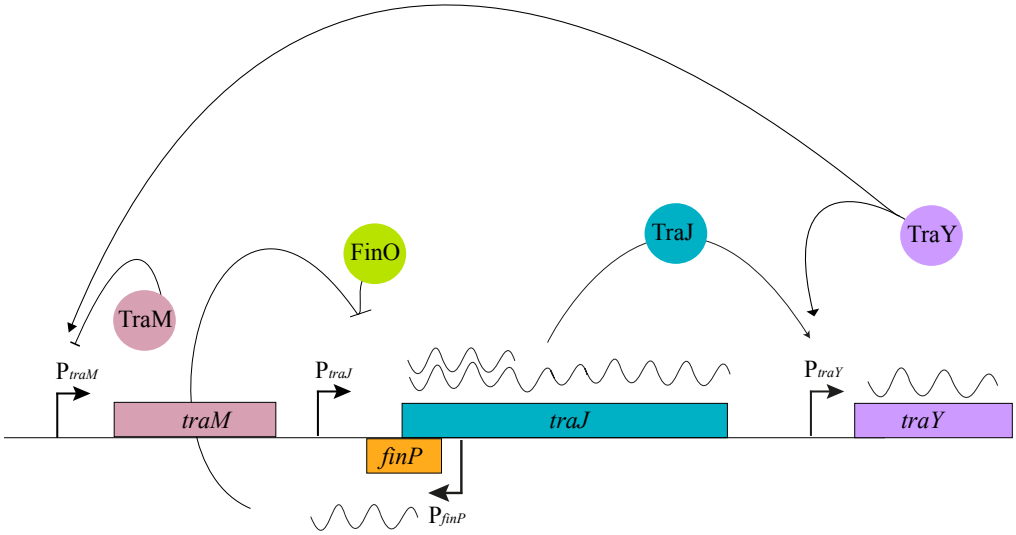

Figure I. 5 Regulatory circuit of the *tra* region. Synthesis of TraJ is controlled by the antisense RNA, FinP (RNA molecules are represented with wave-shaped lines). FinP binds *traJ* mRNA, preventing its translation. TraM binds its own promoter and represses its transcription. Promoter positions and transcription are indicated by straight arrows. Arrows denote activation and blunt lines denote inhibition. Adapted from (Espinosa Alfaro, 2015; Gubbins et al., 2002).

Adhesins

Interaction between the pathogen and host cells is required for colonization. This process is mostly mediated by adhesins on the surface of the microbe. The adhesins are responsible for recognizing and binding to specific receptor moieties of host cells. This interaction involves diverse changes in both the bacterium and the host as a result of the attachment. *S. enterica* has different adhesion systems that have been divided into two categories, non-fimbrial and fimbrial adhesins, which differ in their assembly pathway. Additionally, the flagellum and type III secretion system (TTSS), whose main functions are not involved in adhesion, also contribute to attachment and colonization of host tissues (Soto & Hultgren, 1999).

Non-fimbrial adhesins

This category comprises an heterogeneous group of proteins either secreted via type I secretion systems (SiiE and BapA) or autotransported across the bacterial membranes (ShdA, MisL, SadA) (Wagner & Hensel, 2011).

Fimbrial adhesins

Among the adhesion systems, fimbrial adhesins include the majority of S*almonella* adhesins. Fimbriae are proteinaceous structures extending form the surface of a bacterial cell, and appear in several copies per cell. These structures facilitate adhesion to abiotic and biotic surfaces. Three pathways for the assembly of fimbrial adhesins have been described.

I. Chaperon-usher dependent assembly Pathway (CUP). Fimbrial subunits are directed to the periplasm through the general secretion pathway via an N-terminal secretion sequence that is cleaved off during transport. In the periplasm, chaperones prevent the premature assembly of fimbrial subunits and their degradation by proteases, and direct fimbrial subunits to the usher, which coordinates the assembly of the fimbriae. On the tip of the completed fimbriae, a receptor-binding site interacts with its host receptor. Based on the usher protein (the most conserved fimbrial protein), fimbriae belonging to the chaperone-usher pathway can be divided into 6 groups (γ, κ, π, β, α and σ) (Nuccio & Bäumler, 2007). Several chaperon/usher-type fimbrial operons present in *S. enterica* serotype Typhimurium belong to this group.

II. Nucleator-dependent assembly pathway. The fimbrial subunits are transported into the periplasm via the general secretion pathway. Polymerization of the fimbrial subunits occurs outside of the bacterial cell envelope (Wagner & Hensel, 2011). In *Salmonella*, the fimbrial adhesins assembled through this pathway are designated as "thin aggregative fimbriae" or "curli", and are encoded by the operons *csgDEFG* and *csgBA*. These fimbriae are known to mediate binding to proteins like fibronectin (Olsén et al., 1989) and are involved in biofilm formation (Brombacher et al., 2003; Gerstel & Römling, 2003; Prigent-Combaret et al., 2001)

III. Assembly pathway for type IV fimbriae. In *S. enterica*, type IV pili are only encoded by SPI-7 in the strictly human-adapted serovar Typhi (Zhang et al., 1997; 2000). The subunits for the pilus formation are assembled in the periplasm at the inner membrane, and

are extruded through a secretin in the outer membrane as an intact pilus (Wolfgang et al., 2000).

As it was mentioned before, fimbriae expression involves crosstalk and participates in the coordination of the sequential expression of SPI-1, flagellar and fimbrial systems. Non-fimbrial adhesins are also subject to crosstalked regulation, as it happens with the SPI-4-encoded *sii* operon which is regulated by the SPI-1 SprB regulator (Saini & Rao, 2010).

Hybridization analyses have shown high presence of fimbrial DNA sequences in multiple *Salmonella* serovars. Additionally, each serovar harbors a particular repertoire of fimbrial genes (Townsend et al., 2001). The selective pressure responsible for this uneven distribution is unknown (Humphries et al., 2001) and several tentative explanations have been proposed. On top of their role in attachment, it has been shown the contribution of fimbriae to biofilm formation (Ledeboer et al., 2006). Competitive infection experiments have revealed the participation of fimbriae in long-term colonization in mice (Weening et al., 2005). Each fimbrial operon appears to attach a specific target cell type (Bäumler et al., 1996), enabling the bacterial cell to distinguish among different epithelial cell types. Redundancy of fimbrial tools may counterbalance the loss of a single fimbrial operon, since the inactivation of an individual fimbrial operon results in only moderate attenuation (van der Velden et al., 1998). On the other hand, fimbrial appendages could, at the same time, pose a problem for the bacterium to evade the host immune system, since the fimbrial subunits likely act as antigens during host-pathogen interaction. This aspect could explain why fimbrial expression is commonly regulated by phase variation, generating fimbriated and non-fimbriated subpopulations (Humphries et al., 2001). Phase variation will be described below in more detail.

The *std* fimbrial operon

In *S. enterica* serotype Typhimurium, the elaboration of fimbriae on the surface of cells grown in nutrient broth and their ability to agglutinate yeast or red blood cells, was described in 1966 (Duguid et al., 1966). So far, laboratory-grown cultures of *S. enterica* have only been shown to elaborate type 1 fimbriae (Duguid et al., 1966), encoded by the *fim* operon (Clegg et al., 1987), and thin curled fimbriae (Grund & Weber, 1988; Stolpe et al., 1994), encoded by the *csg* operon (Römling et al., 1998). However, nine additional fimbrial operons, named *bcf*

(Tsolis et al., 1999), *stf* (Emmerth et al., 1999; Morrow et al., 1999), *saf* (Folkesson et al., 1999), *stb*, *stc*, *std*, *sth*, *sti,* and *stj* (McClelland et al., 2001), have been later identified by sequence analysis.

The *std* fimbrial operon was first identified in *S. enterica* serovar Typhi (Townsend et al., 2001) and later found in other *Salmonella enterica* serovars, including Typhimurium (Anjum et al., 2005; Chan et al., 2003; Humphries et al., 2001; Porwollik et al., 2004). However, it has not been detected in related enterobacterial species, suggesting an acquisition mediated by horizontal transfer (Porwollik et al., 2002). The *std* operon belongs to the π-fimbriae group. Members of the same group are well-characterized virulence factors such as pyelonephritis-associated (P) fimbriae of *E. coli* and the Mannose-resistant/*Proteus*-like (MR/P) fimbriae of *Proteus mirabilis* (Nuccio & Bäumler, 2007).

Std fimbriae contribute to cecal colonization by binding to α (1,2) fucose residues, which are abundant in the cecal mucosa (Chessa et al., 2009), facilitating *Salmonella* attachment to the intestinal epithelium. Expression of the *std* operon, as many of the putative fimbrial operons, is tightly repressed under laboratory conditions (Humphries et al., 2003), and derepression occurs in the intestine of infected animals, where Std fimbriae are synthesized. Deletion of *stdAB* results in a competitive defect in colonizing the caecum of mice and in being shed with the faeces (Weening et al., 2005). Results from adhesion assays of *Salmonella enterica* serovar Enteritidis to intestinal epithelial cells as well as to the small intestine and caecum of poultry reveal decreased adhesion of the *stdA* mutant (Shippy et al., 2013).

Recently, the *std* operon (**Figure I. 6**) was defined to contain 6 genes (*stdA-F*), all of them transcribed by the promoter located upstream the first gene (*stdA*; (López-Garrido & Casadesús, 2012)).

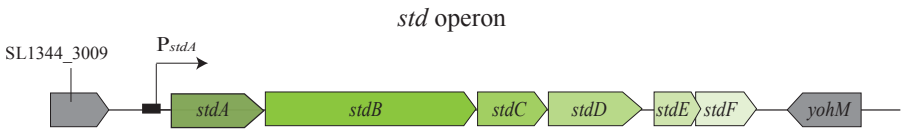

Figure I. 6 Diagram of the *std* operon. *std* operon contains 6 genes (*stdA-F*), all of them transcribed by the promoter located upstream the first gene (P*stdA*).

The mechanisms that prevent *std* expression outside the animal environment remain unknown, but some of the bacterial functions involved in this regulation have been identified. Lack of RosE, an homolog to the *E. coli* ArgR repressor, allows *std* expression under laboratory conditions (Chessa et al., 2008). Dam methylation also prevents expression of the *std* operon, and Dam$^-$ mutants were found among Std-expressing isolates induced by transposon mutagenesis (Chessa et al., 2008). It was previously shown that the GATC-binding protein SeqA is a repressor of the *std* operon, and that the poorly known HdfR protein (a LysR-like protein) is required for *std* expression in *S. enterica* Dam$^-$ mutants (Jakomin et al., 2008). The possibility that HdfR might activate *std* transcription in a Dam-dependent manner is supported by the existence of three GATC sites upstream the *std* promoter, and unpublished results from our group suggest that HdfR binds the *std* promoter in a Dam methylation-dependent manner.

Flow cytometry experiments in Dam$^-$ and SeqA$^-$ mutants show the existence of two subpopulations of Std cells: Std$^+$ and Std$^-$ (Jakomin et al., 2008). The authors suggest the possibility of bistable or phase-variable *std* expression in the animal environment, and speculate that the competition between HdfR and SeqA or RosE might be the responsible for subpopulation formation.

As in other fimbrial operons, structural and regulatory fimbrial proteins are encoded in the same operon. Recently, it was described that the Dam-dependent regulation of SPI-1 requires the last two genes of the *std* operon: *stdE* and *stdF*. Both genes repress SPI-1 expression through HilD at the postranscriptional level (López-Garrido & Casadesús, 2012). Lowered expression levels of the SPI-1 activator HilD would explain the reduced capacity of Dam$^-$ mutants to invade epithelial cells (García-Del Portillo et al., 1999).

HdfR

HdfR is a poorly known protein that belongs to the widely distributed LysR-type family of transcriptional regulators (LTTRs). LTTRs are the largest group of prokaryotic DNA-binding proteins (Maddocks & Oyston, 2008), and act as homodimers or homotetramers (Zaim & Kierzek, 2003). LTTRs control the transcription of multiple operons involved in disparate functions such as amino acid metabolism, oxidative stress, nitrogen fixation, degradation of aromatic compounds, and bacterial virulence (Schell, 1993). Based on the secondary

structure, a helix-turn-helix (HTH) motif has been proposed to conform the DNA-binding domain (Kullik et al., 1995). This type of transcriptional regulators often needs a small ligand acting as a coinducer. The binding site of the inducer has been mapped to the C-terminal part (Lahiri et al., 2009).

Many LTTRs activate the expression of target genes but repress their own expression, frequently by the use of divergent promoters (Zaim & Kierzek, 2003). In *E. coli,* HdfR was shown to negatively control *flhDC* expression through direct binding to its promoter, while *hdfR* expression was negatively regulated by H-NS (Ko & Park, 2000). In *Salmonella* few information is available aside from their role as an activator of *std* transcription in Dam^- and $SeqA^-$ mutants (Jakomin et al., 2008).

RcsCDB phosphorelay system

The Rcs phosphorelay system appears to be conserved in the family *Enterobacteriaceae* (Pescaretti et al., 2009) and it is implicated in gene expresssion of a huge variety of genes, such as those controlling the biosynthesis of colanic acid (Stout & Gottesman, 1990), regulation of flagellum synthesis (Francez-Charlot et al., 2003), motility (Cano et al., 2002) or O-antigen chain length determination (Delgado et al., 2006), among others.

Rcs regulon is activated by cell interaction with surfaces (conditions that lead to biofilm formation) and plays a role in later stages of biofilm formation (Danese et al., 2000; Majdalani & Gottesman, 2005). Perturbation in the cell surface induces the Rcs system. Mutations in *rfa* genes (synthesis of lipopolysaccharide) or *mdo* genes (membrane derived oligosaccharides or periplasmic glucans) implicate induction of Rcs system (Ebel et al., 1997; Majdalani & Gottesman, 2005). In *Salmonella typhimurium*, mutations in *tolB* (periplasmic component of Tol system) were found to increase Rcs regulon activity (Mouslim & Groisman, 2003).

The Rcs system is composed by three proteins: RcsC, RcsD, and RcsB. Environmental signals are transmitted to RcsC, the sensor kinase, which in turn transfers a phosphate to a conserve aspartate on RcsB, the cognate response regulator. RcsD is the intermediary in the phosphoryl transfer (Majdalani & Gottesman, 2005). The main rol of RcsB is as a positive transcriptional regulator of a wide range of genes (capsule synthesis, membrane proteins), but

it has also been reported to behave as a negative regulator (flagellar synthesis) (Francez-Charlot et al., 2003). RcsB may act alone or in combination with other regulators, such as RcsA. RcsA is an unstable regulatory protein that acts with RcsB as an auxiliary regulator in some processes such as the capsule (*cps* loci) synthesis in *E. coli,* where the overexpression of *rcsB* is sufficient to increase *cps* levels in the absence of RcsA (Brill et al., 1988). On the other hand, RcsB is essential, independendly of RcsA levels (Majdalani & Gottesman, 2005).

Dam methylation

Base methylation at specific DNA sequences by DNA methyltransferases is a DNA modification common in all kingdoms of life. In α-Proteobacteria and γ-Proteobacteria, postreplicative N^6 methylation in adenosine moieties lowers the thermodynamic stability of DNA (Engel & Hippel, 1978) and alters DNA curvature (Diekmann, 1987), influencing the interaction between DNA-binding proteins and their cognate DNA sequences (Wion & Casadesús, 2006). The methylation state of such sites can be used as a signal, thereby permitting spatial or temporal control of the interaction between DNA binding proteins and DNA (Messer & Noyer-Weidner, 1988).

In bacteria, two types of DNA methyltransferases perform base modifications: DNA methyltransferases associated with restriction-modification systems (Bickle & Krüger, 1993; Loenen et al., 2014) and solitary DNA methyltransferases (Wion & Casadesús, 2006). Dam methylase of γ-proteobacteria is a solitary methyltransferase.

Dam methylation is found in the orders Enterobacteriales, Vibrionales, Aeromonadales, Pasteurellales, and Alteromonadales (Løbner-Olesen et al., 2005). In *E. coli* and *Salmonella, dam* mutations produce pleiotropic defects but viability is not (or very little) impaired (Torreblanca & Casadesús, 1996). However, in *Vibrio cholerae* and in some strains of *Yersinia* Dam methylation is essential (Julio et al., 2001; Marinus, 1996).

The Dam methylase transfers a methyl group from S-adenosyl-methionine to the N^6 amino group of the adenosine moiety of 5'-GATC-3' sites (Marinus, 1996). Methylation occurs shortly after DNA replication, and methylates transiently hemimethylated GATC sites. Nevertheless, the Dam enzyme can methylate both hemimethylated and nonmethylated GATC sites with similar efficiency (Marinus, 1996). Flanking sequences of the GATC sites

influence the DNA binding ability of the Dam methylase and/or the efficiency of methyl group transfer (Peterson & Reich, 2006).

The Dam protein shares significant identity with DpnII, MboI and other DNA methyltransferases that are part of restriction-modification systems (Low et al., 2001; Løbner-Olesen et al., 2005). This relatedness suggests that Dam has evolved from an ancestral restriction-modification system. An essential difference, however, is that the Dam methylase is highly processive, being able to achieve several methylation reactions without dissociating from the DNA molecule, while restriction-modification methylases are distributive (Urig et al., 2002).

N^6-methyl-adenine is used as a signal for genome defense, DNA replication and repair, nucleoid segregation, regulation of gene expression, control of transposition, and host-pathogen interactions (Low et al., 2001; Løbner-Olesen et al., 2005; Marinus, 1996) (**Figure I. 5**).

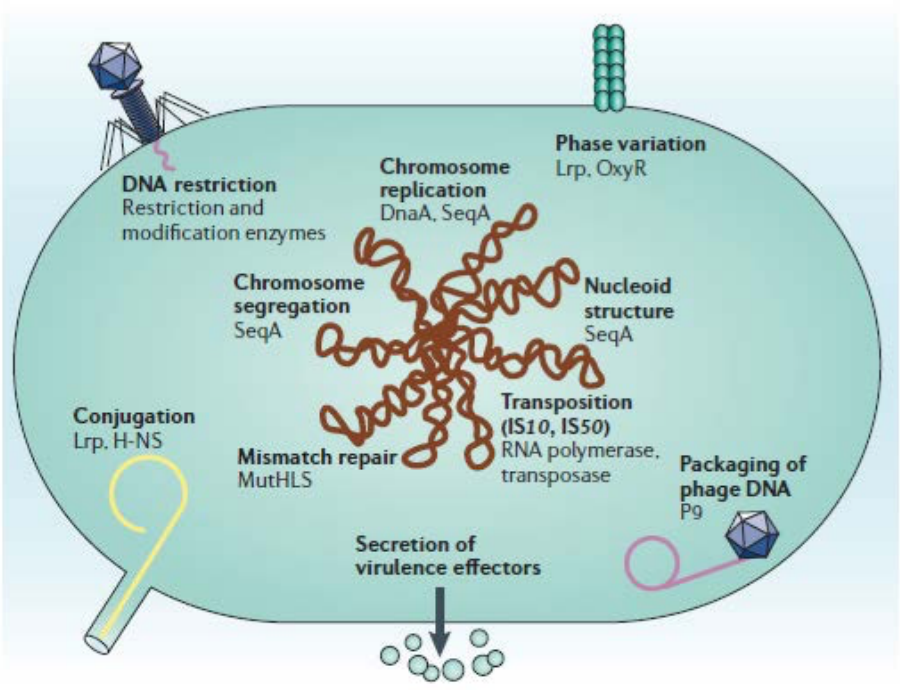

Figure I. 7 Roles of N6-methyl-adenine in enteric bacteria. Methylation-sensitive DNA-binding proteins involved in each process are indicated. Adapted from (Wion & Casadesús, 2006).

Dam methylation plays an important role in the initiation of chromosome replication in *E. coli*. Chromosome replication starts when DnaA, the initiator protein, binds the *oriC*

replication origin, and separates the two strands of the double helix. DnaA binding is only possible if the *oriC* GATCs are methylated; a hemimethylated origin is inactive (Boye et al., 2000). The *oriC* region contains 11 GATC sites in 254 bp, a density ten fold higher than the average expected in the *E. coli* chromosome (Zyskind & Smith, 1992).

The GATC sites of the *oriC* remain hemimethylated for up to one-third of the cell cycle after DNA replication (Campbell & Kleckner, 1990). The protein SeqA avoids further rounds of replication by binding to hemimethylated GATC sites (Taghbalout et al., 2000). SeqA also binds to newly synthesized hemimethylated GATC sites along the chromosome, organizing the daughter chromosomes into nucleoid domains (Han et al., 2003).

During DNA replication, mismatched base pairs errors are common. Dam methylation has an important role in mismatch repair by permitting the identification of the template DNA strand (Hsieh, 2001). The new strand is temporarily nonmethylated. MutS protein recognizes the mismatched base pair and recruits and assembles the MutHLS complex. This assembly activates the endonucleasa activity of MutH, which cleaves the nonmethylated DNA strand at the nearest hemimethylated GATC site, ensuring that the parental template strand is not cut (Friedhoff et al., 2003).

As it was mentioned before, in *E. coli* and *Salmonella, dam* mutations produce pleiotropic defects such as lower colonization capacity, reduced motility, envelope instability, ectopic expression of fimbriae, sensitivity to bile salts, lower expression of virulence genes, and altered LPS O-antigen chain length (Cota et al., 2015; López-Garrido & Casadesús, 2010; Marinus & Casadesús, 2009). These pleiotropic virulence-related defects may explain the extreme attenuation of Dam^- mutants upon oral inoculation in the mouse model (Badie et al., 2007; García-Del Portillo et al., 1999).

Gene regulation by Dam methylation can be divided into two main categories (Low & Casadesús, 2008) :

I. Clock-like controls, in which the methylation state of the DNA is used as a signal to couple gene expression to a specific stage of the cell cycle. Hemimethylation may activate transcription (conjugal transfer gene, *traJ* (Camacho & Casadesús, 2005)) or repress it (chromosome replication gene, *dnaA* (Kücherer et al., 1986).

II. Switch-like controls, in which differential methylation patterns of GATC sites, typically found at or near the promoters of phase variation systems, modulate gene expression (van der Woude, 2011). Binding of proteins to DNA may prevent Dam methylation of certain GATCs, generating hemimethylated GATCs, and nonmethylated GATCs if the block persists during two consecutive DNA replication rounds (Casadesús & Low, 2006). DNA sequences surrounding specific GATCs sites may also decrease the processitivity of Dam methylation (Peterson & Reich, 2006).

Phase variation systems regulated by Dam methylation

As mentioned above, some envelope structures such as fimbriae, flagella or the LPS O-antigen show phase variation and are under epigenetic control (Cota et al., 2015; Cummings et al., 2006; Kingsley et al., 2002; Nicholson & Low, 2000). In phase variation systems, gene expression alternates between active (ON phase) and inactive (OFF phase) states, and often depends on differential DNA methylation patterns (Switch-like controls). Binding of certain transcriptional regulators hinders Dam methylation and creates heritable DNA methylation patterns (**Figure I. 8**).

pap

The *pap* operon encodes pyelonephritis-associated pili that mediate adhesion of uropathogenic *E. coli* to the urinary mucosa (Hernday et al., 2004). Synthesis of Pap pili is subjected to phase variation, and Dam methylation controls the switching between ON and OFF states (Hernday et al., 2002).

The regulatory region of the *pap* operon contains six binding sites for the leucine-responsive regulatory protein, Lrp. Sites 5 and 2 contain GATC motifs known as $GATC^{dist}$ and $GATC^{prox}$, respectively. In the OFF state, Lrp binds cooperatively and with high affinity to sites 1-3 avoiding RNA polymerase binding (Casadesús & Low, 2006; Hernday et al., 2002; 2004). Lrp binding at sites 1-3 reduces the affinity of Lrp for sites 4-6, and preserves the nonmethylated state of $GATC^{prox}$ while $GATC^{dist}$ remains methylated. The high affinity of Lrp for nonmethylated $GATC^{prox}$ and its incapability to bind a methylated $GATC^{dist}$ generates a feedback loop that propagates the OFF state (Hernday et al., 2002; 2004). Switching to the ON state needs translocation of Lrp to sites 4-6. Translocation involves the auxiliary protein

PapI. The PapI/Lrp complex has higher affinity for GATC sites 4-6 than for 1-3 GATCs (Hernday et al., 2003). Binding of Lrp to sites 4-6 hinders methylation of $GATC^{dist}$ and permits methylation of $GATC^{prox}$, favoring the propagation of the ON state (Hernday et al., 2004).

agn43

The *agn43* gene of *E. coli* encodes Agn43, an autotransporter protein whose expression is subjected to phase variation under control of Dam methylation and OxyR (Henderson & Owen, 1999; Henderson et al., 1997). Three GATC sites are contained within the binding site of OxyR at *agn43* regulatory region (Wallecha et al., 2002). OxyR binding to nonmethylated *agn43* GATCs represses transcription and prevents GATC methylation. On the other hand, methylation of GATC sites avoids OxyR binding (Haagmans & van der Woude, 2000; Henderson et al., 1997; Waldron et al., 2002). *agn43* expression thus depends on the competition between OxyR binding and Dam methylation, and switching may occur upon DNA replication (Waldron et al., 2002; Wallecha et al., 2002).

opvAB

The *Salmonella enterica opvAB* operon shows phase variation and creates bacterial lineages with standard ($OpvAB^{OFF}$) and shorter ($OpvAB^{ON}$) O-antigen chains in the lipopolysaccharide. Transcription of OpvAB is controlled by the LysR-type factor OxyR and by Dam methylation. The *opvAB* regulatory region contains four sites for OxyR binding (OBS^{A-D}), and four GATC motifs ($GATC^{1-4}$) which are targets for Dam methylation. $OpvAB^{OFF}$ and $OpvAB^{ON}$ cell lineages present opposite DNA methylation patterns in the *opvAB* regulatory region: (i) in the $OpvAB^{OFF}$ state, $GATC^{1}$ and $GATC^{3}$ are non-methylated, whereas $GATC^{2}$ and $GATC^{4}$ are methylated; (ii) in the $OpvAB^{ON}$ state, $GATC^{2}$ and $GATC^{4}$ are non-methylated, whereas $GATC^{1}$ and $GATC^{3}$ are methylated (Cota et al., 2015).

The OBS^{A} and OBS^{C} sites of *opvAB* are identical to the consensus sequence for OxyR binding while OBS^{B} and OBS^{D} are not. This difference may explain the higher stability of the $OpvAB^{OFF}$ lineage, resulting in a ~600-fold difference in the ON→OFF and OFF→ON transition rates. The predominant OFF state involves binding of OxyR to the OBS^{A} and OBS^{C} sites, which protects $GATC^{1}$ and $GATC^{3}$ from methylation. In this configuration,

GATC^{2} and GATC^{4} are unprotected and therefore are methylated by Dam. In the ON state, OxyR binds to the OBS^{B} and OBS^{D} sites. As a consequence, GATC^{2} and GATC^{4} are protected from methylation and remain non-methylated, while GATC^{1} and GATC^{3} are methylated. RNA polymerase is successfully recruited to the *opvAB* promoter and transcription of *opvAB* takes place. RNA polymerase may contact OxyR and other LysR-type transcription factors within the DNA region occupied by the regulator (Zaim & Kierzek, 2003). Additional factors involved in the formation of OpvAB cell lineages are the GATC-binding protein SeqA (which contributes to the stability of the OpvAB^{OFF} lineage) and the nucleoid protein HU (which contributes to the OpvAB^{ON} lineage) (Cota et al., 2015).

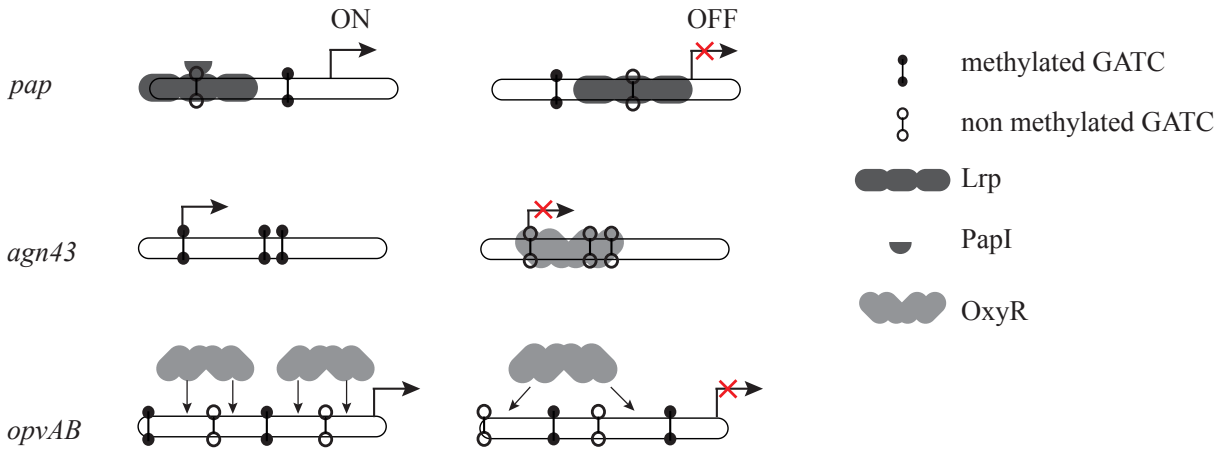

Figure I. 8 Diagrams for Dam methylation-dependent regulation of *pap*, *agn43* and *opvAB* phase variation. For simplicity, binding sites, Dam methylase and RNA polymerase are not represented. Adapted from (Broadbent et al., 2010; Cota et al., 2015).

Phenotypic heterogeneity in bacteria population

Reversible bistability or phase variation involves switching of gene expression from OFF to ON and *vice versa* (van der Woude, 2006; 2011; van der Woude & Bäumler, 2004). Phase variation can be generated by diverse mechanisms, either genetic (homologous or site-specific recombination, slipped strand mispairing at DNA repeat tracks) or epigenetic (DNA methylation) (Bayliss, 2009).

Switching between the ON and OFF states is a stochastic event, but may be modulated by external factors (van der Woude & Bäumler, 2004).

Bimodal gene expression generates phenotypic heterogeneity in the population. This strategy, often observed in loci encoding envelope components or proteins involved in the envelope modification, has been considered a way of avoiding immune responses during animal infection or a way to generate variants with altered ability to colonize niches in the host (van der Woude, 2011; van der Woude & Bäumler, 2004).

The biological significance of bacterial subpopulations can be understood as the consequence of two different strategies: cooperation and bet-hedging (Ackermann, 2013; Lambert et al., 2014; Veening et al., 2008). Cooperation implies that there is an interaction between different phenotypes, so that, in a given environment, both subpopulations together are fitter than any of them separately. Bet-hedging or risk spreading occurs when each subpopulation is fitter than the other in a particular environment, so that the population as a whole is fitter in a variety of conditions and is prepared to adapt to a sudden environmental change.

OBJECTIVES

When this Thesis work started, the literature contained very little information on the *std* operon. The pioneering study that had described the existence of the operon in the genome of *Salmonella enterica* serovar Typhimurium had also reported that *std* expression occurs in the large intestine of animals but not in the laboratory (Humphries et al., 2003). An independent study had shown that repression of *std* expression under laboratory conditions is mainly exerted by DNA adenine (Dam) methylation (Jakomin et al., 2008) and to a lesser extent by a repressor named RosE (Chessa et al., 2008). Transcription of the *std* operon in Dam^- mutants had been shown to require a poorly known LysR-type transcription factor known as HdfR (Jakomin et al., 2008). The same study had raised the possibility that *std* expression might undergo bistability or phase variation. More recently, a study had shown that two products of the *std* operon, StdE anf StdF, downregulated *Salmonella* pathogenicity island 1 (SPI-1) and prevented invasion of epithelial cells (López-Garrido & Casadesús, 2012).

Based on these antecedents, the initial objective of the Thesis (Objective 1) was to investigate whether *std* expression was subjected to phase variation outside the large intestine. Results obtained in this section of the Thesis revealed the existence of Std^{ON} and Std^{OFF} subpopulations outside the animal intestine. Objective 2 was aimed at understanding the molecular mechanisms that control *std* transcription and generate phase variation. Objective 3 pursued the characterization of Std^{ON} state using transcriptomic analysis, chromatin immunoprecipitation, and phenotypic analysis. The main aim of this objective was to determine the extent and physiological significance of StdEF-mediated control of *Salmonella* genes outside the *std* operon. These objectives can be summarized as follows:

Objective 1. Surveys of *std* phase variation in *S. enterica* ser. Typhimurium by single cell analysis.

Objective 2. Molecular analysis of the factors and mechanisms that control *std* transcription and phase variation, with emphasis on the roles of Dam methylation, HdfR, StdE, and StdF.

Objective 3. Characterization of the StdEF regulon of *Salmonella enterica* serovar Typhimurium.

MATERIALS AND METHODS

Bacterial strains and strain construction

Salmonella enterica and *Escherichia coli* strains used in this Thesis are listed in **Table M1**. *Salmonella enterica* strains belong to serovar Typhimurium and derived from the mouse-virulent strain SL1344 or ATCC14028. For simplicity, *Salmonella enterica* serovar Typhimurium is often abbreviated as *S. enterica.*

Table M 1. Strains of Salmonella enterica and E.coli used in this study.

Strain Name	Genotype	Reference
	S. enterica	
SL1344	Wild type	(Hoiseth & Stocker, 1981)
SV5367	SL1344 Δ*dam231*	(Balbontín et al., 2006)
SV8479	SL1344 *stdA::gfp*	This study
SV8480	SL1344 Δ*dam231 stdA::gfp*	This study
SV8481	SL1344 Δ*hdfR stdA::gfp*	This study
SV9311	SL1344 Δ*dam231* Δ*hdfR stdA::gfp*	This study
SV8528	14028 *rcsC11 stdA::gfp*	This study
SV5182	14028 *stdA::lacZ*	(Jakomin et al., 2008)
SV5183	14028 Δ*dam231 stdA::lacZ*	(Jakomin et al., 2008)
SV9312	14028 Δ*dam231 rcsB56N stdA::lacZ*	This study
SV9313	14028 Δ*dam231 rcsC11 stdA::lacZ*	This study
SV9314	14028 Δ*dam231* Δ*tolB stdA::lacZ*	This study
SV9315	14028 Δ*dam231* p-*rcsB stdA::lacZ*	This study
SV5264	14028 Δ*dam231*	(López-Garrido & Casadesús, 2010)
SV5815	14028 Δ*dam231 rcsB56N*	This study
SV9316	14028 Δ*dam231 rcsC11*	This study
SV9317	14028 Δ*dam231* Δ*tolB*	This study
SV9318	14028 Δ*dam231* p-*rcsB*	This study
SV8487	SL1344 Δ*dam231 hdfR*-3xFLAG	This study
SV7889	SL1344 *hdfR::lacZ*	This study

SV9319	SL1344 Δ*dam231 hdfR::lacZ*	This study
SV8188	SL1344 *stdA::lacZ*	This study
SV8449	SL1344 P_{LtetO}*hdfR*	This study
SV8477	SL1344 P_{LtetO}*hdfR stdA::lacZ*	This study
SV9292	SL1344 P_{LtetO}*hdfR stdA::gfp*	This study
SV9123	SL1344 pIC552	This study
SV9124	SL1344 /pIZ2320	This study
SV9125	SL1344 Δ*hdfR* /pIZ2320	This study
SV8192	SL1344 *stdA::lacZ* /pBR328	This study
SV8194	SL1344 *stdA::lacZ* /pIZ2318	This study
SV9320	SL1344 *stdA::lacZ* /pIZ2319	This study
SV8189	SL1344 Δ*hdfR stdA::lacZ*	This study
SV8193	SL1344 Δ*hdfR stdA::lacZ* /pBR328	This study
SV8195	SL1344 Δ*hdfR stdA::lacZ* /pIZ2318	This study
SV9321	SL1344 Δ*hdfR stdA::lacZ* /pIZ2319	This study
SV9322	SL1344 P_{LtetO}*stdEF stdA::gfp*	This study
SV9323	SL1344 Δ*stdEF stdA::gfp*	This study
SV9279	SL1344 Δ*dam231* Δ*stdEF stdA::gfp*	This study
SV9001	SL1344 Δ*dam231* P_{LtetO}*stdEF stdA::gfp*	This study
SV8507	SL1344 P_{LtetO}*stdEF stdA::lacZ*	This study
SV9324	SL1344 Δ*dam231 stdE*-3xFLAG	This study
SV9325	SL1344 Δ*dam231 stdF*-3xFLAG	This study
SV8526	SL1344 ptp166 *stdA::gfp*	This study
SV9205	SL1344 *stdE::gfp*	This study
SV9207	SL1344 Δ*dam231 stdE::gfp*	This study
SV9206	SL1344 Δ*hdfR stdE::gfp*	This study
SV9208	SL1344 Δ*pstdA-stdC stdE::gfp*	This study
SV6503	14028 P_{LtetO}*stdEF* (Cm^r)	(López-Garrido & Casadesús, 2012)
SV6634	14028 P_{LtetO}Δ*stdEF* (Cm^r)	(López-Garrido & Casadesús, 2012)
SV8141	SL1344 P_{LtetO}*stdEF* (Cm^r)	This study

SV8142	SL1344 P_{LtetO}Δ*stdEF* (Cm^r)	This study
SV7553	SL1344 P_{LtetO}*stdEF* (Km^R)	This study
SV7552	SL1344 P_{LtetO}Δ*stdEF* (Km^R)	This study
SV7554	SL1344 P_{LtetO}*stdEF spvA*	This study
SV7555	SL1344 P_{LtetO}Δ*stdEF spvA*	This study
SV7556	SL1344 *spvA::tn5dKm*	This study
SV4938	SL1344 *trg::mudQ* pSLT-	(García-Quintanilla & Casadesús, 2011)
SV7551	SL1344 *traB::lacZ*	This study
SV7550	SL1344 P_{LtetO}*stdEF traB::lacZ*	This study
SV7549	SL1344 P_{LtetO}Δ*stdEF traB::lacZ*	This study
SV8152	SL1344 Δ*ygiD*	This study
SV9326	SL1344 P_{LtetO}*stdEF* Δ*hdfR*	This study
SV9327	SL1344 P_{LtetO}Δ*stdEF* Δ*hdfR*	This study
SV9288	SL1344 *flhC*::*lacZ*	This study
SV9289	SL1344 P_{LtetO}*stdEF flhC*::*lacZ*	This study
SV9290	SL1344 P_{LtetO}Δ*stdEF flhC*::*lacZ*	This study
SV9328	SL1344 Δ*hdfR flhC*::*lacZ*	This study
SV9329	SL1344 P_{LtetO}*stdEF* Δ*hdfR flhC*::*lacZ*	This study
SV9320	SL1344 P_{LtetO}Δ*stdEF* Δ*hdfR flhC*::*lacZ*	This study
SV9331	SL1344 P_{LtetO}*stdEF* Δ*hdfR spvA*	This study
SV9332	SL1344 P_{LtetO}Δ*stdEF* Δ*hdfR spvA*	This study
SV9333	SL1344 Δ*hdfR spvA*	This study
SV8185	SL1344 *sipB*::*lacZ*	This study
SV8186	SL1344 P_{LtetO}*stdEF sipB::lacZ*	This study
SV8187	SL1344 P_{LtetO}Δ*stdEF sipB::lacZ*	This study
SV8109	SL1344 Δf*lhC* sipB::*lacZ*	This study
SV8110	SL1344 P_{LtetO}*stdEF* Δ*flhC sipB::lacZ*	This study
SV8111	SL1344 P_{LtetO}Δ*stdEF* Δ*flhC sipB::lacZ*	This study
SV8103	SL1344 *fliC*::*lacZ*	This study
SV8104	SL1344 P_{LtetO}*stdEF fliC*::*lacZ*	This study

SV8105	SL1344 P_{LtetO}Δ*stdEF fliC*::*lacZ*	This study
SV8106	SL1344 Δ*flhC fliC*::*lacZ*	This study
SV8107	SL1344 P_{LtetO}*stdEF* Δ*flhC fliC*::*lacZ*	This study
SV8108	SL1344 P_{LtetO}Δ*stdEF* Δ*flhC fliC*::*lacZ*	This study
SV8023	SL1344 Δ*flhC traB*::*lacZ*	This study
SV8024	SL1344 P_{LtetO}*stdEF* Δ*flhC traB*::*lacZ*	This study
SV8025	SL1344 P_{LtetO}Δ*stdEF* Δ*flhC traB*::*lacZ*	This study
SV9287	SL1344 P_{LtetO}*stdEF*-3xFLAG	This study
SV9109	SL1344 P_{LtetO}*stdEF* Δ*flhC*	This study
SV9110	SL1344 P_{LtetO}Δ*stdEF* Δ*flhC*	This study
SV9309	SL1344 P_{LtetO}*stdEF* P_{LtetO}*hilD*	This study
SV9310	SL1344 P_{LtetO}Δ*stdEF* P_{LtetO}*hilD*	This study
SV9113	SL1344 P_{LtetO}*stdEF invH::lacZ*	This study
SV9114	SL1344 P_{LtetO}Δ*stdEF invH::lacZ*	This study
SV9119	SL1344 P_{LtetO}*stdEF* P_{LtetO}*hilD invH::lacZ*	This study
SV9120	SL1344 P_{LtetO}Δ*stdEF* P_{LtetO}*hilD invH::lacZ*	This study
SV9273	SL1344 Δ*dam231* Δ*stdEF*	This study
SV7890	SL1344 P_{LtetO}*stdEF hdfR::lacZ*	This study
SV7891	SL1344 P_{LtetO}Δ*stdEF hdfR::lacZ*	This study
TR5878	*galE496* r(LT2)$^-$ m(LT2)$^-$ r(S)$^+$ *ilv-542 metA22 trpB2* Fels2$^-$ *fliA66 strA120 xyl-404 metE551 hspL56 hspS29*	J.R. Roth
	E. coli	
DH5α	supE44 ΔlacU169 (Φ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 reA1	(Hanahan, 1983)
BL-21	F$^-$ *dcm ompT hsdS* (rB- mB-) *gal* (malB+) K-12 (λS)	Stratagene
	BL-21 pET28a-*stdE*	This study

Targeted gene disruption was achieved by Datsenko and Wanner method (Datsenko & Wanner, 2000). This method is based in the λ Red recombination system. The strategy consists in replacing the chromosomal sequence by an antibiotic resistance marker (contained in pKD3, pKD4 or pKD13) that is generated by polymerase chain reaction (PCR) using oligonucleotides that harbor 40 nucleotides of homology with the sequence to be replaced. λ Red recombination gene expression is carried out under an inducible promoter inside a thermosensitive low copy number plasmid (pKD46).

Cultures harbored pKD46 plasmid (this plasmid expresses the λ Red system under the *araB* promoter which is inducible by arabinose) grown in LB with ampicillin at 30ºC were diluted 1:100 into LB with ampicillin and arabinose 1 mM and incubated in a shaker at 30ºC until $O.D._{600} \sim 0.8$. The competent cells were prepared and electroporation was done as further described.

After selection, antibiotic resistance cassettes introduced during strain construction can be excised by recombination with plasmid pCP20 (Datsenko & Wanner, 2000). pCP20 plasmid can be transduce with P22. This transduction is incubated at 30ºC for 1h and spread in LB with ampicillin. To eliminate the plasmid, green plates were prepared without antibiotic and incubated at 37ºC. To confirm the excision of the marker, the strains were streaked in plates of LB ampicillin and plates of LB with chloramphenicol or kanamycin. The excision of the antibiotic marker was checked by colony PCR with external oligonucleotides.

The oligonucleotides used for gene disruption (named as *UP* and *DO*) and allele verification (named as *E1* and *E2*) by the polymerase chain reaction (PCR), are listed in **Table M2**.

For construction of *lac* fusions in the *Salmonella* chromosome, FRT sites generated by excision of Km^R cassettes with pCP20 (Datsenko & Wanner, 2000) were used to integrate either pCE37 or pCE40 (Ellermeier et al., 2002).

For strain construction operations involving chromosomal markers we used transduction with P22 HT 105/1 *int201* ((Schmieger, 1972) and G. Roberts, unpublished). The recipient strain was transduced using a P22 lysate from a strain with the desirable genetic marker. All the markers used in this thesis were selected directly by spreading the transduction mixture on selective media. Transduction protocol is described below.

Table M 2. Oligonucleotides used in thi study.

Oligonucleotide name	sequence (5'→3')
stdA129GFP UP	tttcactggtaccatcaccaactcaccctgtgatatcgcataagaaggagatatacatatg
stdAGFP DO	ccgtggacggcttctccctgtcgttatttaccgcgtgaaattatcacttattcaggcgta
stdA E1	ggaaagttcaggtgcttcg
stdAE2	gctttcggtgttgtcgtcc
P_{LtetO}*hdfR UP*	atatcgattagtcgtcgtaaagctttccgccatcctgcacaggcttacccgtcttactgtc
P_{LtetO}*hdfR DO*	ggaaagtttttaacaattccgtatccactgtgctctccacaaaacctctccataactggg
hdfR E1	ggagagcacagtggatacgg
hdfR E2	gattatctgatcaggtaatc
promotorhdfRBglII FOR	agctagatctccgtgacgagagaatccacg
promotorhdfRXhoI REV	agctctcgaggatacgaaagctcaccgcgg
P_{LtetO}*stdEF pStd UP*	gttttctgctgcaatacccgttactgttacctataactaaaggcttacccgtcttactgtc
P_{LtetO}*stdEF pStd DO*	tcagggcacataaaacctctccataactgggtaaatgatgtgctcagtatctctatcactgatag
stdE21GFP UP	ccagttatggagaggttttatgtgccctgataatacacactaagaaggagatatacatatg
stdEGFP DO	ttaccgacccggcgttttgataccagcggcggtccggcttttatcacttattcaggcgta
stdE E1	tgctgcaatacccgttactg
stdE E2	caggctgcctgtatgcg
P_{LtetO} *sense*	ttggaacctcttacgtgcc
mutpStdA-stdC UP	ccttttacttgcattatatccagttttttatgctaccagtgtgtaggctggagctgcttc
mutpStdA-stdC DO	gatgtcgcccctgcgtccttaaacgcgcagttcagggcatcatatgaatatcctccttag
Km P_{LtetO}*UP*	ttaatgtcatgataataatggtttcttagacgtcgatatcgtgtaggctggagctgcttc
Km P_{LtetO}*DO*	cttggattctcaccaataaaaaacgcccggcggcaaccgacatatgaatatcctccttag
NdeIstdE-FOR	ttttcatatgtgccctgataatacaca
EcoRIstdE-REV	ttttgaattcttatgttccgtcatcctcatcc
std-P_{LtetO}*UP*	attcacccgcttataattac
std southern DO	cagctaatgcctaatcagttg

EMSA-std300 FOR	attcacccgcttataattac
EMSA-std735 FOR	cgcattaatatcccccagcc
EMSA-std REV	attacgcatagataatatgtc
8032 EMSA-Fwd Pdps	gcgctattacttcgtc
8033 EMSA-Rev Pdps	cgggatccctcatatcctcttgatgtttgtgt
pBR328-Fw	actgtccgaccgctttgg
pBR328-Rv	gccagcaaccgcacctg
ygiD UP	ggttatctgagttcttcctctgtgaagaaagcgtatggtggtgtaggctggagctgcttc
ygiD DO	cgccatcgggcaatcattcagcgccttatccggcctaccccatatgaatatcctccttag
ygiD E1	tatcaatttgccgtccgaacc
ygiD E2	tcgaaggatagattctcctg
RT-hilD FOR	agtttgctttcggagcggta
RT-hilD REV	agcaccaacatcccaggttc
RT-promotorstd FOR	cattaaaaagtatttctttgatg
RT-promotorstd REV	gaaaattcttattcaaattaaaac
RT-flgE DIR	gacggtacgacaacgaacac
RT-flgE REV	aaaaccgttctggctaatcg
RT-hilA DIR	gaatcttttcatggctggtca
RT-hilA REV	gggtccaattttaaacactcgt
RT-motA DIR	taggggcgttcattgtcg
RT-motA REV	acgggatagctttcatcgtg
RT-sipB DIR	gtaatggtggccgatgaaat
RT-sipB REV	cgcctgctgaataaacgac
RT-traA DIR	gtacggtcaaggcgacattt
RT-traA REV	ccgcgagaataacccactt
RT-trg DIR	cggggtcgtacaaacgat
RT-trg REV	gcggtaatttccgagattttt

RT-hdfR DIR	ttggcgttatattgcagcag
RT-hdfR REV	ccagccgcagataattgagt
RT-cheA DIR	cggtgatgtcgattcgtatg
RT-cheAREV	tctacctgcttgcccagttt
RT-cheM DIR	gaaggttcggatgcgattta
RT-cheM REV	acgggaagagaggtcggtat
RT-cheB DIR	gctgctcagttcggaaaaac
RT-cheB REV	gcacatgtcggatagcttca
RT-stdA DIR	catcaccaactcaccctgtg
RT-stdA REV	aaatctgtccaaacggaacg
RT-rfaH DIR	tcagccattttgtgcgctt
RT-rfaH REV	tttaggatcgacaacgcctt

Construction of the relevant strains

For the construction of the strains SV8479 (*stdA::gfp*) and SV9205 (*stdE::gfp*), a fragment containing the promoterless green fluorescent protein (*gfp*) gene and the chloramphenicol resistance cassette was PCR-amplified from pZEP07 (Hautefort et al., 2003) using the pair of oligonucleotides *stdA129GFP UP* and *stdAGFP DO*; and *stdE21GFP UP stdEGFP DO* respectively. The fragment was integrated into the chromosome of *S. enterica* using the Lambda Red recombination system (Datsenko & Wanner, 2000), and verified with the pair of oligonucleotides *stdA E1* and *stdA E2*; or *stdE E1* and *stdE E2*. *stdA::gfp* and *stdE::gfp* transcriptional fusions were formed defective in StdA and StdE synthesis, since only the first 129nt and 21nt of the genes are kept in the construct.

The construction of the strains SV8141 (SL1344 P_{LtetO}*stdEF*) and SV8142 (SL1344 P_{LtetO}Δ*stdEF)* was performed by transduction of *S. enterica* SL1344 cells using lysates from the previously described strains SV6503 (14028 P_{LtetO}*stdEF*) and SV6634 (14028 P_{LtetO}Δ*stdEF*) (López-Garrido & Casadesús, 2012). In both constructions, the P_{LtetO}promoter is inserted upstream *stdE* on the *Salmonella* chromosome. Insertion of the P_{LtetO} promoter removed the upstream genes in the *std* operon and the native promoter upstream *stdA*. A chloramphenicol resistance cassette (Cm^R) is linked to the P_{LtetO} promoter in these cells. For

mating experiments described below, the resistance cassette was changed from Cm^R to kanamycin (Km^R). To do so, the Km^R cassette (including the FRT sites) was amplified from pKD4 (Datsenko & Wanner, 2000), with oligos *KmP_{LtetO} UP* and *KmP_{LtetO} DO*. The resulting PCR product was transformed into SL1344 P_{LtetO}*stdEF* and SL1344 P_{LtetO}Δ*stdEF* cells containing the pKD46 plasmid, and positive clones were selected by their ability to grow in a kanamycin containing medium. Finally, the Km^R cassette introduced during construction was excised by recombination with plasmid pCP20 (Datsenko & Wanner, 2000).

The same P_{LtetO} insertion upstream *stdEF* gene was performed but maintaining the upstream genes in the *std* operon together with the native promoter upstream *stdA*. To do so, we used the oligonucleotides *P_{LtetO}stdEF pStd UP* and *P_{LtetO}stdEF pStd DO* and verified with *P_{LtetO} sense* and *stdE E2*. Strains SV9322, SV9001 and SV8507 were generated by transduction.

Strain SV8449 (P_{LtetO}*hdfR*) contains the P_{LtetO} promoter upstream *hdfR* coding region on the *Salmonella* chromosome. P_{LtetO} promoter was used in order to achieve a constitutive and moderate expression of *hdfR*. To construct this strain, the P_{LtetO} promoter was amplified from SV7553 (SL1344 P_{LtetO}*stdEF* (Km^R)) with oligos *P_{LtetO}hdfR UP* and *P_{LtetO}hdfR DO*. The resulting PCR product was integrated into the chromosome of *S. enterica* using the Lambda Red recombination system (Datsenko & Wanner, 2000) by transformation into SL1344 cells containing the pKD46 plasmid, and positive clones were selected by their ability to grow in a kanamycin containing medium. Finally, the Km^R cassette introduced during construction can be excised by recombination with plasmid pCP20 (Datsenko & Wanner, 2000). Insertion of the P_{LtetO} promoter removed the native promoter of *hdfR* avoiding transcriptional regulation of this gene.

Bacteriophages

Bacteriophage P22 H5 is a virulent derivative of bacteriophage P22 that carries a mutation in the *c2* gene (SMITH & H, 1964) , and was kindly provided by John R. Roth, University of California, Davis. For simplicity, P22 H5 is abbreviated as P22.

Culture media and growth conditions

Luria-Bertani broth (LB) was used as standard rich medium (10 g/l tryptone, 5 g/l yeast extract and 10 g/l NaCl). Solid media contained agar at 1.5% final concentration. Green plates (Chan et al., 1972) called EBU plates as well, were used to discard the presence of lysogenic isolates after transductions are made of LB medium supplemented with 10 ml/l K_2HPO_4 25%, 5 ml/l glucose 50%, 2.5 ml/l fluorescein 1% and 1.25 ml/l Evans blue 1%.

Salmonella enterica cultures were grown usually at 37ºC, and exceptionally at 30ºC (strains containing the thermolabile pKD46 or pCP20 plasmids). Liquid cultures were shaken at 200 rpm for aeration. Any other conditions of growth would be indicated.

Antibiotics were used at the final concentrations described previously (Prieto et al., 2004) and listed below, in **Table M. 3**, together with other chemicals employed, as the 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; Sigma-Aldrich) used as indicator for monitoring β-galactosidase activity in plate test.

Table M 3. Final concentration of antibiotics and other chemicals used in this Thesis.

Chemical	Final concentration
Antibiotics	
Ampicillin (Ap)	100 μg/ml
Chloramphenicol (Cm)	20 μg/ml
Kanamycin (Km)	50 μg/ml
Tetracycline (Tet)	5 μg/ml
Other chemicals	
EGTA	0.004 μg/ml
X-gal	40 μg/ml

Bacterial transduction

P22 lysates

To prepare P22 lysates, 4 ml of P22 stock (100 ml NB (3 g/l meat extract and 5 g/l peptone), 2 ml E50x (300 g/l $H_3C_6H_5O_7 \cdot H_2O$, 14 g/l MgSO4, 1965 g/l $K_2HPO_4 \cdot 3H_2O$, 525 g/l $NaNH_4HPO_4 \cdot H_2O$), 1ml glucose 20% and 0.1 ml P22 phage) was mixed with 1 ml of the donor strain. The mixture was incubated at 37ºC and 200 rpm for 4-8 h. Bacterial debris was removed by centrifugation for 10 min at 4,500 rpm. The supernatant was recovered in a glass fresh tube, and 800 µl of chloroform were added and vortexed. The lysates were maintained at room temperature for a few hours and then stored at 4ºC.

Transduction in liquid medium

100 µl of an overnight culture of the recipient strain and 10-20 µl of the lysate of the donor strain were mixed in a sterile 1.5 ml tube. This mixture was incubated at 37ºC (pCP20 transduction is incubated at 30ºC) and 200 rpm for 30-60 min (depending on the marker to be transduced). The mixture was spread on selective plates that were incubated at 37ºC until colonies appeared.

Detection of lysogenic transductants

Transductants harboring a selective marker could have been infected by P22 phage and become pseudolysogenic (the *int* mutation avoids integration, and delays the formation of true lysogens). As time goes on, pseudolysogens become resistant or immune to new P22 infections and cannot be lysed or transduced again. Pseudolysogeny should thus be avoided. To obtain phage-free isolates, transductants were purified by streaking on green plates (with antibiotics if necessary), prepared according to Chan *et al.* (Chan et al., 1972). On these plates, pseudolysogens are dark colored and P22-free colonies are light colored. This color difference is due to cell lysis in the pseudolysogenic colony, which causes acidification of the medium and turning of the pH indicator, darkening the agar. A transductant was considered P22- free when streaking did not give rise to any dark colony.

P22 sensitivity assay

In EBU plates, isolates that form light colour colonies could be lysogens that do not undergo visible lysis. These isolates are P22-resistant and can be mistaken by real P22-free isolates. To avoid this situation, an assay to detect P22-sensitive strains is advisable. A streak with a P22 H5 lysate is done on an LB or EBU plate, and air-dried. The test strain is then streaked in a perpendicular way to the H5 streak. P22-sensitive strains grow until they reach the H5 streak, while P22-resistant strains grow over the streak.

DNA manipulation and transfer

Plasmids

Plasmids used in this study are listed in **Table M4.**

Table M 4. Plasmids used in this study.

Plasmid	Description	Reference
	S. enterica	
pCP20	*bla cat* cI857 λP_R *flp* pSC101 oriTS, Ap^R, Cm^R	(Cherepanov & Wackernagel, 1995)
pIC552	*galK' lac*$^+$, Ap^R	(Macián et al., 1994)
pKD3	*bla* FRT *cat* FRT PS1 PS2 oriR6K, Ap^R, Cm^R	(Datsenko & Wanner, 2000)
pKD4	*bla* FRT *aph* FRT PS1 PS2 oriR6K, Ap^R, Km^R	(Datsenko & Wanner, 2000)
pKD13	*bla* FRT *aph* FRT PS1 PS4 oriR6K, Ap^R, Km^R	(Datsenko & Wanner, 2000)
pKD46	*bla* P_{BAD} *gam bet exo* pSC101 oriTS, Ap^R	(Datsenko & Wanner, 2000)
pZEP07	pZEP06 derivative, t0T1, Cm^R	(Hautefort et al., 2003)
pET28a	vector used to construct 6His fusions ,Ap^R	Novagen
pTP166	Cloned *dam* gene	(Marinus et al., 1984)
p*rcsB*	pUHE2-21 *lacIq* containing *rcsB* gene	(Pescaretti et al., 2009)
pIZ2318	pBR328*std*	This work
pIZ2319	pBR328*std*Δ*stdF*	This work
pIZ1991	pET28a-*stdE*-6His	This work
pIZ2320	pIC552-*hdfRpromoter region*, Ap^R	This work

For construction of pIZ1991, a DNA fragment containing *stdE* coding region was amplified using oligonucleotides *NdeIstdE-FOR* and *EcoRIstdE-REV* and cloned into pET28-a (Novagen) using the NdeI and EcoRI sites.

Plasmid pIC552 is a promoter-less vector used to construct transcriptional l*acZ* fusions; in this plasmid, the *lacZ* ORF is preceded by the translational start site of *galK* (Macián et al., 1994). The *galK* ribosomal binding sites and this adjacent region guarantee a high efficiency of the lacZ translation, so that the β-galactosidase activity is directly proportional to the transcriptional activity of the fragment cloned. The suspected promoter region of the *hdfR* gene was amplified using the oligonucleotides *promotorhdfRBglII FOR* and *promotorhdfRXhoI REV* listed in **Table M2**, and clone into pIC552 plasmid, generating the plasmid pIZ2320 listed in **Table M4**.

Extraction of plasmid DNA

Commercial system GenElute TM Plasmid Miniprep Kit, provided by Sigma-Aldrich Co (St. Louis, Missouri, USA) was used for the extraction of plasmid DNA.

Extraction of genomic DNA

For the extraction of genomic DNA, 5 ml of cells from a stationary culture ($O.D._{600}$~2) were collected and re-suspended in 0.4 ml of buffer lysis (50 mM Tris-HCl pH8, 10 mM EDTA, 100 mM NaCl and 0.2% SDS), 4 µl of RNAse (10 mg/ml) were added and the mixture was incubated at 37ºC for 30 minutes. After that, 20 µl of proteinase K (20 mg/ml) was added and it was incubated for 2 h at 65ºC. Finally, were performed 3 or 4 extractions with phenol:chloroform-isoamyl alcohol in a 2:1 proportion. Optionally, one extraction with chloroform:isoamyl alcohol (24:1) can be performed. DNA was precipitated al -20º C by adding 1/10 volume of sodium acetate 3 M and 2.5 volumes of ethanol. After precipitation, genomic DNA was washed with 70% ethanol and re-suspended in 50 µl of water.

Digestion, modification and ligation of DNA fragments

Restriction endonucleases were supplied by Roche Diagnotics GmbH (Indianapolis, Indiana, USA), New England Biolabs (Beverly, Massachusetts, USA) and Promega Biotech (Madison, Wisconsin, USA). Enzymes were used following the manufacturer's instructions.

For ligation of DNA fragments with the appropriated vector (properly digested), 1 U of T4 DNA ligase (1 U/µl, Roche Diagnosstics) was used in the buffer supplied by the manufacturer. Routinely, the mixture was incubated at 16ºC overnight.

Electrophoresis in an 0.8% agarose (Low Electro Endosmosis agarose-Pronadisa, Conda, España) gel made up in TAE 1x buffer (40 mM Tris-acetate and 10 mM EDTA pH7.7) was used to test the quality of DNA extraction, to determine DNA fragments after plasmid restriction, to estimate the efficiency of endonuclease restriction, etc. The loading buffer used was 10X Loading buffer supplied by Takara. The 1 Kb ladder (GIBCO BRL, Life Technologies, New York, USA) was used as molecular weight marker. Ethidium bromide (0.5 µg/ml final concentration) was added to the gels to make bands visible. Gels were illuminated with UV and pictures were taken with Gel Doc^{TM} Imager- BioRad.

Bacterial transformation

High efficiency *E. coli* transformation

Competent cells were prepared using a variation of the Inoue method (Inoue et al., 1990), which guarantees high transformation efficiency ($5x10^7$ to $5x10^8$ transformants per µg of plasmid DNA). An *E. coli* DH5α/BL-21 overnight culture was diluted 100-1000 times in 200 ml of SOB (20 g/l tryptone, 5 g/l yeast extract, 0.5 g/l NaCl, 0.19 g/l KCl, adjust with NaOH to pH7 and 5 ml $MgCl_2$ 2 M after autoclaving), and incubated at 22ºC and 200 rpm until OD_{600} ~0.5 was reached. The culture was chilled quickly on ice and kept on ice for 10 minutes. Cells were harvested by centrifugation at 2,500 g and 4ºC for 10 min. The pellet was resuspended in 20 ml of cold filtrated TB (10 mM PIPES, 15 mM $CaCl_2$, 250 mM KCl, adjust to pH6.7 with KOH, 55 mM $MnCl_2$), and 1.5 ml of dimethyl sulfoxide (DMSO) was added. After 10 min incubation on ice, aliquots of 0.2-0.5 ml were prepared, freezed in liquid nitrogen, and stored at -80º C.

For transformation, an aliquot of competent cells was slowly thawed on ice and was mixed with the plasmid. The mixture was incubated on ice for 30 minutes, subjected to heat shock (42^{o}C, 45 s), and cooled on ice for 1 min. One ml of LB was then added. The mixture was incubated at 37^{o}C for 1 h; finally, the cells were concentrated in 100 µl and spread on selective media.

Salmonella electroporation

An overnight culture was diluted 1/100 in LB and, depending on the strain, was grown at 37°C or 30°C (strains with a thermolabile plasmid like pKD46) until an OD_{600} ~0.8 was reached. The cultured was chilled on ice and kept on ice for 5 minutes. 20 ml cells were harvested by centrifugation at 4000 rpm for 5 min at 4°C. The supernatant was discarded and the bacterial pellet was softly re-suspended in 1 ml of cold $_{dd}H_2O$. Once cells were re-suspended, 19 ml of cold water were added, and cells were washed again. Finally, cells were harvested and re-suspended in 250 µl of water.

Electroporation was performed by mixing 1 µl of plasmid DNA or 10 µl of PCR product with 50 µl of competent cells. The mixture was transferred to a cuvette that had 2 mm gap between the plates. The cuvette was subjected to an electric discharge in the electroporator (2.5 KV, 200 Ω and 25 µF). The electroporator employed was a BTX Electrocell Manipulator 600 (Harvard Apparatus, Holliston, Massachusetts, USA). After the discharge, 1 ml of LB was added to the cells, and the mixture was transferred to a 10 ml tube, which was incubated at 37° C with shaking for 1 h. Finally, cells were concentrated in 100 µl and spread on selective media.

pBR328-based plasmid library genetic screen

pBR328-based multicopy plasmid library of the *Salmonella enterica* genome was used to search for factors that might activate expression of the *std* operon in a wild type (Dam^+) background *stdA::lacZ* strain (SV5182).

This library was previously constructed and described (López-Garrido & Casadesús, 2012). Briefly, *Salmonella enterica* Serovar Typhimurium SL1344 was partially digested with Sau3A. DNA fragments ranging between 7-11 kb long were ligated to the pBR328 vector, previously digested with BamHI and dephosphorylated. *Salmonella* strain TR5878 was transformed with the ligation products, and ampicillin–resistant clones were selected on LB with Ap plates. Pools of approximately 1000 independent transformants were collected and lysed with phage P22 HT 105/1 *int201*. As a quality control, the ability of the library pools to complement null mutations in *araA* (required for growth with L-arabinose as the sole carbon source) or *xylA* (required for growth with D-xylose as the sole carbon source) were tested.

Lysates that permitted successful complementation were stored and used for plasmid delivery to recipient strains in subsequent genetic screens.

The genetic screen was done by mixing 100 µl of a saturated *stdA::lacZ* culture with 10 µl of each P22 lysate of the pBR328 plasmid library pools. After 45 minutes, the transductions were plated in LB Cm and X-gal agar plates and incubated at 37ºC overnight. 10 transductions were made for each of the 9 different pools (90 transductions total). The following day, the *stdA::lacZ* background colonies with increased X-gal activity were culture in LB Cm liquid medium. Minipreps were done to recover the pBR328 plasmid derivatives. Positive plasmids were re-transformed into SV5182 to corroborate the positive phenotypes. β-galactosidase activity of the selected candidates was tested and the plasmids were sent for Sanger DNA sequencing of the inserts using pBR328 specific oligonucleotides that flanked the region of the insertion. pIZ2318 and pIZ2319 obtained from the genetic screen are listed in **Table M4**.

β-galactosidase assays

Levels of β-galactosidase activity were assayed using the $CHCl_3$-sodium dodecyl sulphate permeabilization procedure (Miller, 1972). β-galactosidase activity data are the averages and standard deviations from ≥3 independent experiments.

Motility assays

Motility assays were carried out in motility agar plates, containing 10 g/l tryptone (Difco), 5 g/l NaCl, and 0.25% Bacto-agar (Cano et al., 2002). A sterile stick was soaked in saturated bacterial cultures grown in LB, and used to inoculate motility agar plates. Bacterial motility halos were compared after growth at 37ºC for 6 hours.

Phenotypic assays for biofilm formation

Salmonella strains (SL P_{LtetO}*stdEF*, SL P_{LtetO}Δ*stdEF* and SL Δ*ygiD*) were tested for their ability to produce biofilm in LB medium (Latasa et al., 2012). The culture was grown at 22ºC

for 7-10 days in static. For better visualization biofilm was stained with a 0.1% solution of crystal violet.

Matings

Cultures of the donor and the recipient were grown overnight in LB broth. Cells were harvested by centrifugation and washed with LB. 1 ml aliquots of both strains, were sucked onto a membrane filter 0.45 mm pore size. The donor/recipient ratio was 1:1. The filters were placed on LB plates and incubated during 4h at 37ºC in GasPak microaerophilic jars (Camacho & Casadesús, 2002) and microaerophilic conditions were obtained by a GENbox Anaer bag, supplied by BioMériex (Marcy l'Etoile, France). An anaerobic indicator was used to monitor microaerophilic conditions. Filters were place in 10 ml tubes with 1 ml of fresh LB and vortex for recovering the cells. Serial dilutions were done before plating. Conjugation frequencies were calculated per donor cell, as previously described (Camacho & Casadesús, 2002; García-Quintanilla et al., 2008).

Subcellular fractionation

Subcellular fractionation was performed as previously described (Pucciarelli et al., 2002), with some modifications. Briefly, bacteria were grown in LB medium at 37ºC and spun down by centrifugation at 15,000 g for 5 min at 4ºC, then resuspended twice in cold phosphate-buffered saline (PBS pH 7.4, 0.137 M NaCl, 2,7 M KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_20$, 1.4 mM KH_2PO_4). Unbroken cells were further removed by low-speed centrifugation (5,000 g, 5 min, 4ºC). The supernatant was centrifuged at high speed (100,000 g, 30 min, 4ºC) and the new supernatant was recovered as the cytosol fraction. The pellet containing envelope material was suspended in PBS with 0.4% Triton X-100 and incubated for 2 h at 4ºC. The sample was centrifuged again (100,000 g, 30 min, 4ºC) and divided into the supernatant containing mostly inner membrane proteins and the insoluble fraction corresponding to the outer membrane fraction. An appropriate volume of Laemmli buffer (SB4x: 0.000125% bromophenol blue, 200 mM Tris-HCl pH 6.8, 20% β-mercaptoethanol, 40% glycerol and 8% SDS) was added to each fraction. After heating (100ºC, 5 min) and clearing by centrifugation (15,000 g, 5 min, room temperature), the samples were analysed for protein content by

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) described by Laemmli (Laemmli, 1970).

Protein extracts and Western blotting analysis

Total protein extracts were prepared from bacterial cultures grown at 37ºC in LB medium until stationary phase (O.D.600 ~2). A volume containing ~2.5 x 10^8 cells were collected by centrifugation and suspended in 50 µl of Laemmli sample buffer (SB4x: 0.000125% bromophenol blue, 200 mM Tris-HCl pH 6.8, 20% β-mercaptoethanol, 40% glycerol and 8% SDS). Proteins were resolved by SDS-PAGE (Laemmli, 1970) using 12% gels. Conditions for protein transfer have been described elsewhere (Jakomin et al., 2008). Primary antibody was anti-Flag M2 monoclonal antibody (1:5,000, Sigma-Aldrich, St. Louis, MO). Goat anti-mouse horseradish peroxidase-conjugated antibody (1:5,000; Bio-Rad, Hercules, CA) was used as secondary antibody. Proteins recognized by the antibody were visualized by chemoluminescence using the luciferin–luminol reagents of Supersignal West Pico Chemiluminiscent Substrate (Thermo Scientific, Waltham, MA, USA) and developed in a LAS3000 mini system (Fujifilm, Tokyo, Japan).

Purification of StdE protein and antibody generation

A DNA fragment containing *stdE* was amplified using oligonucleotides *StdEprotUP* and *StdEprotDO,* and cloned into pET28a (Novagen) using the NdeI and EcoRI sites. The recombinant plasmid (pIZ1991) was verified by restriction analysis and DNA sequencing. For 6×His-StdE purification, plasmid pIZ1991 was transformed into *E. coli* BL-21. BL-21/pIZ1991 was grown in LB broth containing Kanamycin, and expression of 6×His-StdE was induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). After 3 h of induction, cells were centrifuged and resuspended in 10 ml of lysis buffer (20 mM Tris, 300 mM NaCl, 10 mM imidazole) per g of pelleted cells, and were lysed by sonication. The suspension was centrifuged at 10,000 rpm for 20 min at 4º C and the supernatant containing the soluble fraction of 6×His-StdE was transferred to a HisTrap HP nickel affinity chromatography column (GE Healthcare, Wauwatosa, WI, USA). The column was washed 3 times with 4 ml of washing buffer (20 mM $NaH_2PO_4 \cdot H_2O$, 0.5 mM NaCl, 30 mM imidazole). Protein elution was performed with 3 ml of elution buffer (20 mM $NaH_2PO_4 \cdot H_2O$, 0.5 mM NaCl, 300 mM

imidazole). Aliquots of each fractios were loaded in SDS-PAGE gel and stain with Coomassie Brilliann Blue R-250 (Sigma-Aldrich) solution (0.25% (w/v) Coomassie Brilliant Blue R-250, 10% (v/v) acetic acid and 10% (v/v) ethanol). Elution fractions enriched in 6×His-StdE were selected. Imidazole was removed by dialyzing in cellulose membranes with PBS 1X. Purified 6×His-StdE protein was sent to Biomedal S.L (Sevilla, Spain) for polyclonal antisera production in rabbits. Working dilution was made based on manufacture recommendations.

RNA isolation

To prepare cells for RNA extraction, 3 ml of fresh LB was inoculated with a 1:100 dilution from an overnight bacterial culture, and incubated with shaking at 200 rpm in an Infors Multritron shaker at 37ºC. A 2 ml aliquot from a stationary culture ($O.D._{600}$~2) was centrifuged at 13000 rpm, 4ºC, during 5 min. The pellet was resuspended in 100 µl of a solution of lysozyme (3 mg/ml in water; Sigma Chemical Co.). Cell lysis was facilitated by a freeze-thaw cycle. After lysis, RNA was extracted using 1 ml of TRIsure reagent following manufacture's instructions (Bioline, Taunton, Massachusetts, USA). Total RNA was resuspended in 30 µl of RNase-free water, and subsequently clean by extraction with acidic phenol, followed by a second extraction with chloroform:isoamilic alcohol (24:1). After extraction, RNA was precipitated with ethanol and 3 M sodium acetate, and the dried pellet was resuspended in RNase-free water. The quantity and quality of the RNA was determined using a ND-1000 spectrophotometer (NanoDrop Technologies).

Quantitative reverse transcriptase PCR (qRT-PCR)

For qRT-PCR, *Salmonella* RNA was extracted from stationary phase cultures ($O.D._{600}$~2) as described above, and the concentration was determined using a ND-1000 spectrophotometer (NanoDrop Technologies). An aliquot of 1 µg of RNA was used for cDNA synthesis using QuantiTec® Reverse Transcription Kit (Quiagen) following manufacturer's instructions. Quantitative RT-PCR reactions were performed in a Light Cycler 480 II apparatus (Roche). Each reaction was carried out in a total volume of 10 µl on a 480-well optical reaction plate (Roche) containing 5 µl SYBR mix, 0.2 µl DYE II (Takara), 4 µl cDNA (1/10 dilution) and two gene-specific primers at a final concentration of 0.2 mM each. Real-time cycling

conditions were as follows: (i) 95ºC for 10 min and (ii) 40 cycles at 95ºC for 15s, 60ºC for 1min. A non-RT control was included for each primer set. Triplicates were run for each reaction, and the Ct value is averaged from them. Absence of primer dimers was corroborated by running a dissociation curve at the end of each experiment to determine the melting temperature of the amplicon. Melting curve analysis verified that each reaction contained a single PCR product. Gene-specific primers were designed with ProbeFinder software (http://www.universalprobelibrary.com) from Roche Applied Science and are listed in **Table M2**.

For quantification, the efficiency of each primer pair was determined to be between 90%-110%, following the instructions for efficiency determination described in the "Guide to Performing Relative Quantification of Gene Expression Using Real-Time Quantitative PCR" (Applied Biosystems). These efficiencies indicate that the amount of DNA is doubled in each PCR cycle, and allows for direct comparison between different genes. Relative RNA levels were determined using the ΔΔCt method as described in the above mentioned guide. Briefly, each gene *Ct* value is normalized to the *Ct* value for the internal control (*rfaH*), which gives the ΔCt value. This value is then related to a given gene in the reference strain (*S. enterica*, in this case) giving us the ΔΔCt value. Since the amount of DNA doubles in each PCR cycle, the relative amount of input cDNA can be determined by using the formula $2^{-\Delta\Delta Ct}$. Each ΔΔCt determination was performed at least in three different RNA samples (three biological replicates), and the results are representative example of such determinations.

Analysis of *hilD* mRNA decay

Use of quantitative RT-PCR to monitor mRNA decay has been previously described (Baker et al., 2007). An overnight LB culture of the strain under study was diluted 100 fold, and incubated at 37ºC with shaking until $O.D._{600}$~2). Transcription initiation was stopped by adding rifampicin (500 mg/ml). Cultures were kept at 37ºC and aliquots were extracted at 1 min intervals. Each aliquot was immediately immersed in liquid N_2 and kept frozen until RNA extraction. RNA was extracted using the standard protocol above described and amounts of *hilD* mRNA were determined with qPCR, using oligos *RT-hilD FOR* and *RT-hilD REV* as described above. Three independent qRT-PCR reactions were performed.

Southern blot

Genomic DNA was isolated as previously described from stationary cultures in LB ($O.D._{600}$ ~2). Forty μg of each DNA sample were digested with SspI (New England Biolabs, Ipswich, MA), purified and divided into four fractions, three of which were subsequently digested with DpnI, MboI or Sau3AI (New England Biolabs). After digestion the samples were run in a denaturing 8% TBE-polyacrilamide (19:1) 8 M urea gel. Electrophoresis was carried out in a Hoefer SE400 (Hoefer Scientific Instruments, San Francisco, California) subjected to an electric field of 35 mA for 60 minutes.

DNA was transferred to an Amersham Hybond-N+ membrane (GE Healthcare, Wauwatosa, WI, USA) using a semidry Electroblotting system (Thermo Scientific, Waltham, MA, USA). The DNA in the membrane was then immobilized by UV crosslinking. A radioactive probe was prepared by PCR using dCTP [α-32P] (Perkin Elmer, Waltham, MA) and oligonucleotides *stdP*$_{LtetO}$UP and *std southern DO*. After the PCR reaction, non-incorporated nucleotides were removed by treatment in a Sephadex G-25 column (Illustra MicroSpin G-25 columns, GE Healthcare, Wauwatosa, WI) following manufacturer's instructions. Prior to hybridization the double-stranded DNA probe was denatured by heating at 95ºC for 3 min, followed by incubation on ice. Hybridization with the probe was performed overnight at 52ºC in hybridization buffer (0.5 M sodium phosphate pH 7.2, 10 mM EDTA, 7% SDS). Excess probe was removed with washing buffer (40 mM sodium phosphate pH 7.2, 1% SDS) at 48ºC (three washes, 30 min each). The membrane was developed using a FLA-5100 Scanner (Fujifilm, Tokyo, Japan).

Microarray procedures and data analysis

Salmonella enterica comparative transcriptomic analyses were performed in a strain that constitutively expressed *stdEF* (SL1344 P$_{LtetO}$*stdEF*- SV8141) and in a control strain carrying an in-frame deletion of both genes (SL1344 P$_{LtetO}$Δ*stdEF*- SV8142). RNA from two biological replicates of each strain was isolated and used for the assay.

Transcriptomic analyses were performed using *Salmonella enterica* serovar Typhimurium SL1344 4X72K array, custom prepared for Dr. Antonio Juarez (Paytubi et al., 2014). Hybridation and microarray scanning were performed at the Functional Genomics Core of

the Institute for Research in Biomedicine, Baldiri Reixac, Barcelona, Spain (http://www.dnaarrays.org/). Normalization of the expression signals was done with RMA (Irizarry et al., 2003) using Partek Genomics suite6.5 (6.11.0207). Raw transcriptomic data were deposited at the Gene Expression Omnibus, G.E.O, database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE45488. Raw transcriptomic data was analysed building the ratio between the two conditions of interest by subtracting. Those genes with a difference higher than 4-fold in expression were further study.

Chromatin Immunoprecipitation followed by sequencing (ChIP-seq) and data analysis

ChIP-seq assays were used to determine chromosome-wide DNA binding profiles of StdE and StdF in *Salmonella enterica* serovar Typhimurium SL1344. P_{LtetO}*stdEF*-3xFLAG strain (SV9287) was used to perform ChIP-seq experiments. 20 ml of fresh LB was inoculated with a 1:100 dilution from an overnight bacterial culture, and incubated with shaking at 200 rpm in an Infors Multritron shaker at 37ºC. Cells collected at $O.D._{600}$ ~2 were crosslinked with 1% formaldehyde (37% solution) at 37ºC for 25 min, following by quenching the unused formaldehyde with 450 mM glycine for 5 min. Crosslinked cells were harvested by centrifugation at 9000 rpm at 4ºC for 10 min and washed with 10 mL of TBS pH7.6 (2.42 g/l Trizma base, 8 g/l NaCl). The washed cells were re-suspended in 1 mL of lysis buffer (10 mM Tris-HCl pH 8, 20% sucrose, 50 mM NaCl, 10 mM EDTA) and after an additional centrifugation step, the cells were re-suspended in 0.5 mL of lysis buffer with lysozyme (20 mg/ml in water; Sigma Chemical Co.). The cells were incubated for 30 min at 37°C and then treated with 4 mL of IP buffer (50 mM HEPES-KOH pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na deoxycholate, 0.1% SDS and 1mg/mL phenylmethylsulfonyl fluoride (PMSF). The lysate was then sonicated using a Bioruptor (Diagenode) with 5 cycles of 7 minutes (30″ON/30″OFF) at high intensity. Cell debris was removed by centrifugation at 13000 rpm at 4°C for 20 min and the resulting supernatant was used as cell extract for the immunoprecipitation. The range of the DNA size resulting from the sonication procedure was 100 - 500 bp, and the average DNA size was 300 bp.

To immunoprecipitate StdE-DNA and StdF-DNA complexes, 800 µL of chromatin, 20 µL of Ultralink Immobilised protein A/G beads (Pierce) and 2 µL of the corresponding antibody

were used. Control sample (mock-IP) was performed with no antibody added. Four samples were used for each antibody and four samples for the control. They were then incubated for 90 minutes at room temperature on a rotating wheel. Beads were transferred to a Spin-X column tube (Costar) and centrifuge at 3000 rpm for 1min. Beads were gently re-suspended in 500 µL of IP buffer and incubated on wheel for additional 3 min. This step was done twice. Beads were washed with 500 µL of IP salt buffer (50 mM HEPES-KOH pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na deoxycholate, 0.1% SDS, 1mg/mL phenylmethylsulfonyl fluoride (PMSF) and 500 mM NaCl), IP wash buffer and TE pH 8.0 (10 mM Tris-HCl and 1 mM EDTA) by re-suspending and re-centrifuging sample. The column was transferred to a fresh tube and the beads were re-suspended in 100 µL of elution buffer (50 mM Tris-HCl at pH 7.5, 10 mM EDTA, and 1% SDS) and incubated at 65ºC for 20 min. After centrifuging at 3000 rpm for 1 min the flow-through was treated with 10 µL of 40 mg/mL of a mixture of proteases (Pronase, Roche) made up in TBS pH7.6 (2.42 g/l Trizma base, 8 g/l NaCl). The samples were heat at 42ºC for 2h and 65ºC for 6 hours. The reactions were then kept at 4ºC overnight. The samples were clean up by using a PCR clean up Kit (Promega) and re-suspended in 50 µL of H_2O.

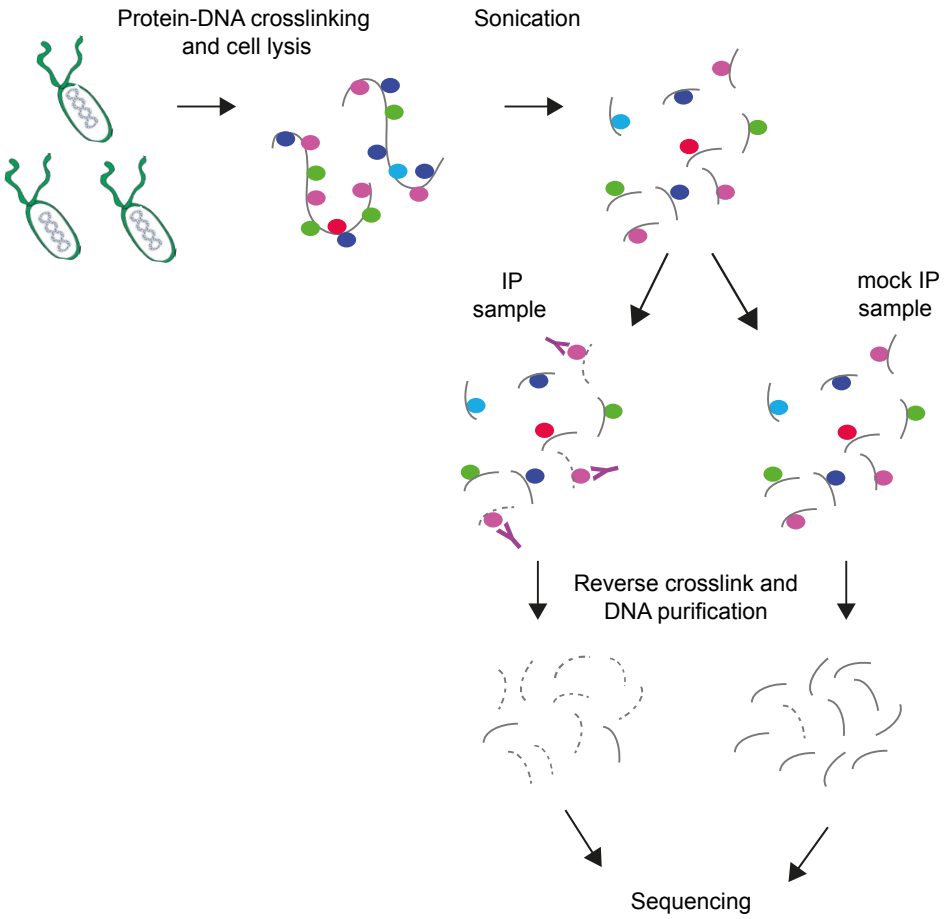

Figure M. 1 Chromatin Immunoprecipitation (ChIP). The diagram sums up the Chromatin Immunoprecipitation process and the final enrichment of the IP sample in the desired DNA fragments (dashed line).

Input and ChIP DNA samples were sent for sequencing at the Functional Genomics Core Facility of the Institute for Research in Biomedicine, Barcelona (Spain). Next generation sequencing was carried out using Illumina's sequencing technology. Ultra DNA Library Prep Kit (Illumina) was used for library preparation. Libraries were sequenced on Illumina's Genome Analyzer II system. 50 nucleotides single end reads were obtained strictly following manufacturer's recommendations. Illumina sequencing data were pre-processed with the standard Illumina pipeline version 1.5.

BAM files reported by the sequencing facility of the Functional Genomics Core Facility were converted to FASTQ format with the BAM2FASTQ tool (http://www.hudsonalpha.org/gsl/information/software/bam2fastq). The quality of the sequence reads was examined using FASTQC (Andrews, 2010) that reported the presence of Illumina adapters. The adapters were trimmed with the FASTX_CLIPPER tool of the

FASTX-Toolkit suite (http://hannonlab.cshl.edu/fastx_toolkit/). Reads shorter than 40 nt were discarded.

NCBI GCA_000210855.2 genome assembly of S.enterica SL1344 was used as reference genome. Mapping was performed with Bowtie (Langmead et al., 2009) allowing only two-mismatches for unique alignment.

Peaks were called using CisGenome version 2.0 (Ji et al., 2008) using default parameters. The IGV browser (Thorvaldsdóttir et al., 2013) was used for data visualization. Genes closest to a ChIP peak were identified using the bedtools suite (Quinlan & Hall, 2010). Peak boundaries sequences were extracted from the reference genome using the fastaFromBed utility from the BEDTools suite (Quinlan & Hall, 2010).

Chromatin Immunoprecipitation followed by quantitative PCR (ChIP-qPCR)

ChIP-qPCR assays were used to check StdE, StdF and HdfR binding to the *std* promoter region. SL1344 Δ*dam stdE*-3xFLAG, SL1344 Δ*dam stdEF*-3xFLAG and SL1344 Δ*dam hdfR*-3xFLAG strains were used to perform ChIP-qPCR experiments. The Chromatin Immunoporecipitation protocol used was previously described for ChIP-seq assay. After DNA purification, quantitative PCR was performed following the protocol previously described and using specific oligonucleotides *RT-promotorstd FOR* and *RT-promotorstd REV* for the amplification of the *std* promoter region in both IP sample and mock IP sample.

Inmunofluorescence microscopy

Cells from 1.5 ml of an exponential culture (OD_{600}~ 0.5) of *Salmonella enterica* SL1344 were collected by centrifugation, washed, resuspended in 1ml TE buffer and fixed by adding the same volume of cold 70% ethanol. Ethanol-fixed cells (100 µl) were stained with polyclonal rabbit anti-StdA serum (Baumler's lab) (1:250). After extensive PBS + Gelatin 0.02% washing, a goat anti-rabbit antibody conjugated to (Fluorescein isothiocynate) FITC (1:500) was used. Inmunostained cells were stained with Hoechst 33258, 1.5 mg ml^{-1}, in 10µl mounting medium (40% glycerol in 0.02 M phosphate buffered saline, pH 7.5). 20µl of

ethanol-fixed cells was spread onto a poly-L-lysine-coated slide, and dried at room temperature. Slides of stained samples were stored at room temperature in the dark. Images were obtained by using an Olympus IX-70 Delta Vision fluorescence microscope (Olympus, Tokyo, Japan) equipped with a 100X UPLS Apo objective. Pictures were taken using a CoolSNAP HQ/ICX285 camera (Roper Technologies, Sarasota, FL) and analysed using ImageJ software (Wayne Rasband, Research Services Branch, National Institute of Mental Health, MD).

Flow cytometry

Bacterial cultures were grown at 37ºC in LB until exponential ($O.D._{600}$ ~0.4) or stationary phase ($O.D._{600}$ ~2). Cells were then diluted in PBS to a final concentration of ~10^7/ml. Data acquisition and analysis were performed using a Cytomics FC500-MPL cytometer (Beckman Coulter, Brea, CA). Data were collected for 100,000 events per sample, and were analysed with CXP and FlowJo8.7 software. Data are shown either by a dot plot (forward scatter [cell size] *vs* fluorescence intensity) or histograms (% cell counts *vs* fluorescence intensity).

Statistical analysis

The Student's *t* test was used to determine if the differences in our experiments were statistically significant.

RESULTS

Chapter I: Characterization of the *std* operon

Expression of the *std* operon

It has been described that the *std* fimbrial operon is tightly repressed in *Salmonella enterica* in a Dam-dependent manner (Balbontín et al., 2006). Dam$^-$ mutants show high expression of the *std* operon (Balbontín et al., 2006), and this expression is dependent on the HdfR protein (Jakomin et al., 2008). Moreover, bimodal expression for this operon has been suggested (Jakomin et al., 2008), since the authors observed two subpopulations in Dam$^-$ and SeqA$^-$ backgrounds.

To further understand the expression conditions of the *std* operon, and based on the possibility of heterogeneous expression suggested by Jakomin et al., 2008, single cell analysis of *stdA* expression was performed using an *stdA::gfp* transcriptional fusion. This fusion is defective in StdA synthesis, since only the first 129 nt of the *stdA* gene are present in the construction. However, the downstream genes of the operon are expressed since the construct does not contain a transcriptional terminator downstream of the *gfp* gene. Flow cytometry assays were performed using strains *stdA::gfp*, Δ*dam231 stdA::gfp* (non-polar deletion of *dam*), Δ*dam231* Δ*hdfR stdA::gfp*, Δ*hdfR stdA::gfp*. A representative experiment is presented in **Figure C1. 1**.

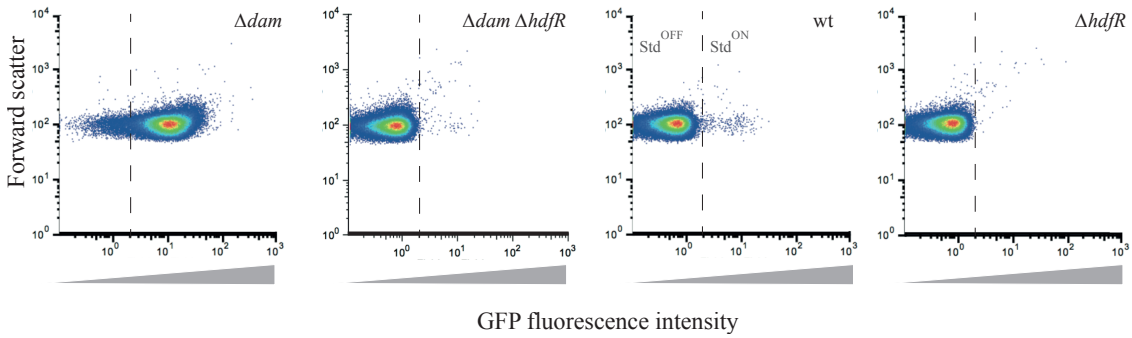

Figure C1. 1 Regulation of the Dam-dependent *std* operon and role of HdfR. GFP fluorescence intensity in a strain carrying an *stdA::gfp* fusion in different genetic backgrounds (Δ*dam*, wt, Δ*hdfR)*. The Dam$^-$ mutant shows bimodal expression of the *stdA::gfp* fusion and the expression is dependent on HdfR, as described by (Jakomin et al., 2008). In a wild type background, a minor subpopulation (0.3% of the cells, Std^{ON}) appears to express *stdA::gfp*. The subpopulation disappears in a HdfR$^-$ background, underlining the essential role of HdfR in *std* regulation.

The experiment shows that *std* expression is repressed by Dam methylation in a HdfR-dependent manner as previously described. Just ~ 0.3 to 3% of cells produce *stdA::gfp* in a

wild type background. As observed for expression in a Dam$^-$ background, this expression is also dependent on HdfR. This observation indicates that expression of the *std* operon occurs only in a fraction of the population under laboratory conditions, suggesting bistable expression of the operon.

Also, upon labeling Std fimbriae and under the fluorescence microscope, only a small fraction of *Salmonella enterica* wild type cells appeared to be Std-frimbriated (**Figure C1. 2**). Bacteria were labelled with rabbit anti-StdA serum (obtained from A. Baümler's lab) and goat anti-rabbit IgG FITC conjugate for the detection of Std fimbriae. This observation, along with previous studies of phase variation of fimbrial operons (Hernday et al., 2004; Humphries et al., 2001; Norris et al., 1998), supports the idea that the *std* fimbrial operon undergoes heterogeneous expression.

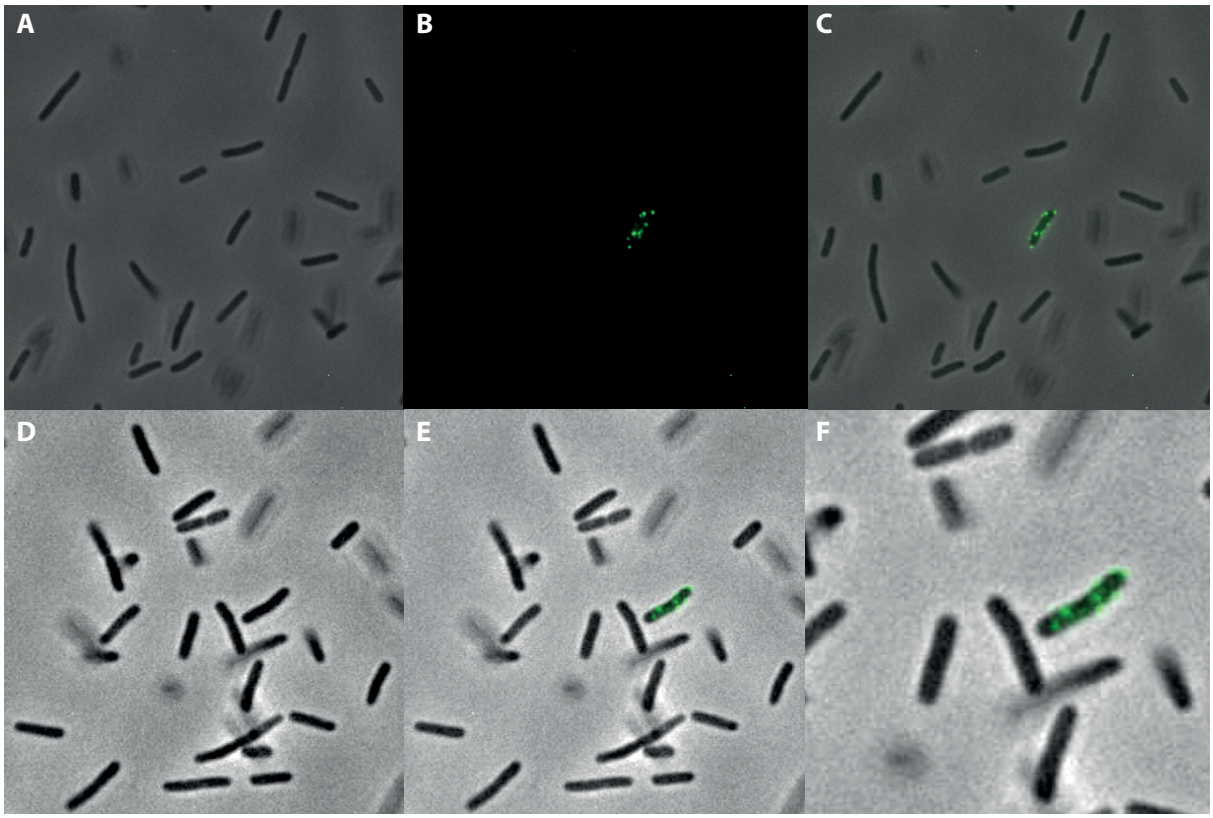

Figure C1. 2 Detection of StdA fimbriae in *Salmonella enterica.* Immunofluorescence microscopy for the detection of StdA fimbriae in *Salmonella enterica* in a wild type background. Left column (panels A and D) shows bacterial cells of *Salmonella enterica* (Hoechst stain). Panel B shows StdA detection with anti-StdA antiserum and goat anti-rabbit antibody conjugated to FITC (green signal). In panels C, E and F both channels were merged.

Both flow cytometry and fluorescence microscopy approaches reveal that expression of *stdA* (and in consequence, expression of *std* operon) occurs in a small fraction of the population. Based on flow cytometry results, the Std^{ON} subpopulation varies from 0.3 to 3% of the population depending on the growth phase of the cells. However, no expression was detected when using the *stdA::lacZ* fusion on X-gal plates, despite the fact that the *stdA::lacZ* fusion behaves as expected in a Dam^- background (Δ*dam stdA::lacZ*), showing blue colonies on X-gal plates.

Transcriptional regulation of the *std* operon

The mechanisms that prevent expression of the *std* operon outside the animal intestine are not well understood. It is already well-known that several factors repress *std* expression, such as Dam methylation (Balbontín et al., 2006; Chessa et al., 2008), RosE, an homolog of *E. coli* ArgR, and the GATC-binding protein SeqA (Chessa et al., 2008). On the other hand, the poorly known HdfR protein (a LysR-like factor) is required for *std* expression in *S. enterica* Dam^- mutants (Jakomin et al., 2008), and it is also needed for *std* expression in a wild type background.

During this Thesis, we had the chance of working in collaboration with the group of Dr. Mónica Delgado, INSIBIO, CONICET-UNT, Argentina. This group is interested in the regulatory mechanism of the signal transduction system RcsCDB. Previous results showed that a RcsC11 mutant undergoes constitutive activation of the RcsCDB system, and this activation leads to attenuation of *S. typhimurium* virulence (Costa & Antón, 2001; Mouslim & Groisman, 2003; Mouslim et al., 2003; 2004), partially through the repression of *hilA*, *invF*, *sipC* and *invG* genes (Delgado et al., 2006; Mouslim et al., 2004). Unpublished data from Delgado's group reveals that the *rcsC11* strain has a defective phenotype of adhesion to Caco-2 cells. These observations led us to speculate about a potential relation between constitutive expression of *rcsB* and *std* operon expression.

In order to study whether the RcsCDB system controls *std* operon expression, we used the chromosomal *stdA::gfp* transcriptional fusion mentioned above. The *gfp* fusion was introduced into an RcsC11 background, and the distribution of GFP activity during exponential growth (O.D ~0.8) was determined.

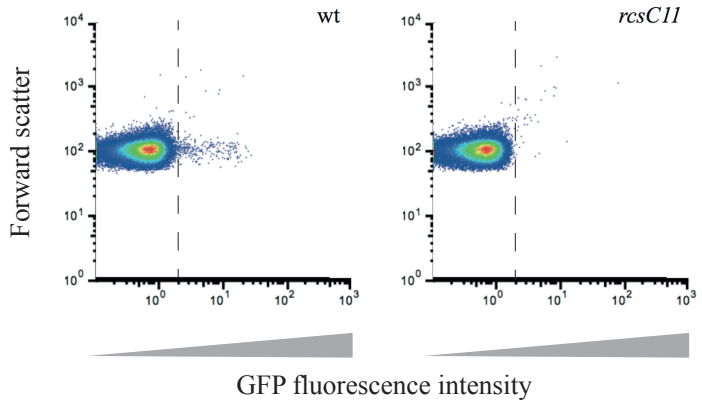

Figure C1. 3. Distribution of *stdA::gfp* activity in wild type and *rcsC11* backgrounds. The std^{ON} subpopulation disappears in the strain *rcsC11 stdA::gfp,* indicating repression of *std* when *rcsB* is constitutively expressed.

As observed in **Figure C1. 3**, constitutive expression of *rcsB* (in a RcsC11 mutant background) eliminates the Std^{ON} subpopulation, indicating that RcsB represses *std* expression. In order to further understand the regulatory mechanism that controls *std* expression through *rcsB*, the β-galactosidase activity assay of the *stdA*::*lacZ* translational fusion was monitored in different Dam^- genetic backgrounds. Note that Dam^- background was chosen in order to have enough *stdA::lacZ* expression. From the results summarized in **Figure C1. 4**, we can conclude that constitutive expression of *rcsB* (RcsC11 mutant) ends up in a 10-fold reduction of *stdA::lacZ* expression in the Dam^- mutant.

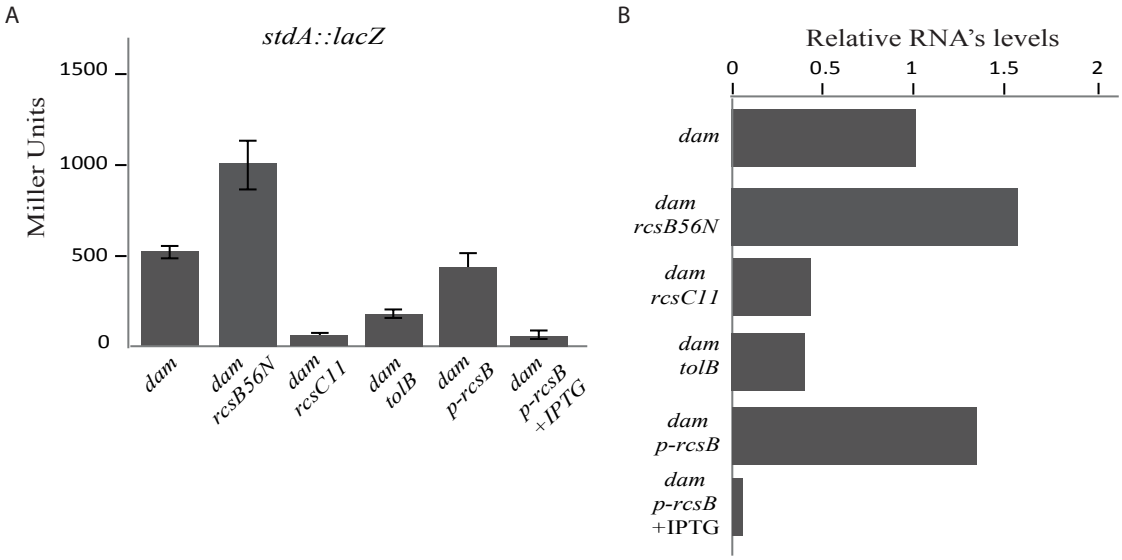

Figure C1. 4 Regulation of *stdA::lacZ* expression by RcsB A. β-galactosidase activity of *stdA::lacZ* translational fusion in different genetic backgrounds. **B.** Relative mRNA levels of *stdA* in different genetic backgrounds**.** RNA levels were determined for at least 3 biological replicates and a representative experiment is shown.

In contrast, inactivation of RcsB (the *rcsB*$_{D56N}$ strain contains a point mutation in the *rcsB* gene that inactivates RcsB by a single amino acid change) in a Dam$^-$ background increases *stdA*::*lacZ* expression two fold compared with the Dam$^-$ mutant (**Figure C1. 4A**).

Previous reports from Dr. Delgado's group demonstrated that the RcsCDB system is activated by a *tolB* mutation (TolB is involved in the integrity of cell envelope) and by *rcsB* overexpression from the p*rcsB* plasmid (Mouslim & Groisman, 2003, Pescaretti et al., 2010). As we expected, the β-galactosidase activity levels obtained in *dam tolB* and *dam* p*rcsB* (+ IPTG to overexpress *rcsB*) strains were similar to those observed in the *dam rcsC11* (**Figure C1. 4A**). When IPTG was not added to the culture medium of the *dam* p*rcsB* strain, the β-galactosidase activity levels produced were similar to those observed in the Dam$^-$ mutant (**Figure C1. 4A**). To further confirm these results, transcription of *stdA* was monitored by qRT-PCR. As shown in **Figure C1. 4B**, the results correlate well with those obtained by monitoring β-galactosidase activity, and the *stdA* expression levels in *dam rcsC11*, *dam tolB* and *dam* p*rcsB* (+ IPTG) strains were significantly lower than those observed in the Dam$^-$ mutant. Moreover, the *stdA* transcription levels of the Dam$^-$ RcsB$_{D56N}$ double mutant were higher than those observed in the Dam$^-$ mutant. These results, together with the observations presented above, indicate that the RcsCDB system downregulates *stdA* gene expression, both in wild type and Dam$^-$ backgrounds.

In addition, we studied whether *std* downregulation is due to specific binding of RcsB on the *std* promoter region. For this purpose, we first carried out a bioinformatic analysis using the *std* promoter region to identify a putative RcsB-binding site. The architecture of the *std* promoter including the GATC sequences, -10 and -35 modules, and the +1 transcription site was described by Jakomin et al. (2008) (**Figure C1. 5A**). As shown in **Figure C1. 5A**, a consensus RcsB-binding site, already described in other RcsB-regulated genes (Carballès et al., 1999; Mouslim & Groisman, 2003; Wehland & Bernhard, 2000), was found overlapping the -35 box of the *std* regulatory region.

These findings suggest that RcsB binding to the -35 box may prevent binding of RNA polymerase, thereby inhibiting transcription of *std*. Binding of RcsB to this region was further confirmed by Dr. Delgado`s group, showing that migration of 300 pb and 735 bp PCR products (generated with *EMSA-std300 FOR and EMSA-std REV; and EMSA-std735 FOR* and *EMSA-std REV* pair of oligonucleotides respectively), both containing the *std* promoter and the putative RcsB-binding site, were retarded in Electrophoretic Mobility Shift Assay

(EMSA) with increasing RcsB concentration (Farizano, JV, personal communication). A 234 bp DNA fragment was used as a negative control and, as expected, no retardation was observed (**Figure C1. 5B**). Altogether, these observations indicate that RcsB is a repressor of *std* expression, and that it acts by direct binding to the *std* promoter.

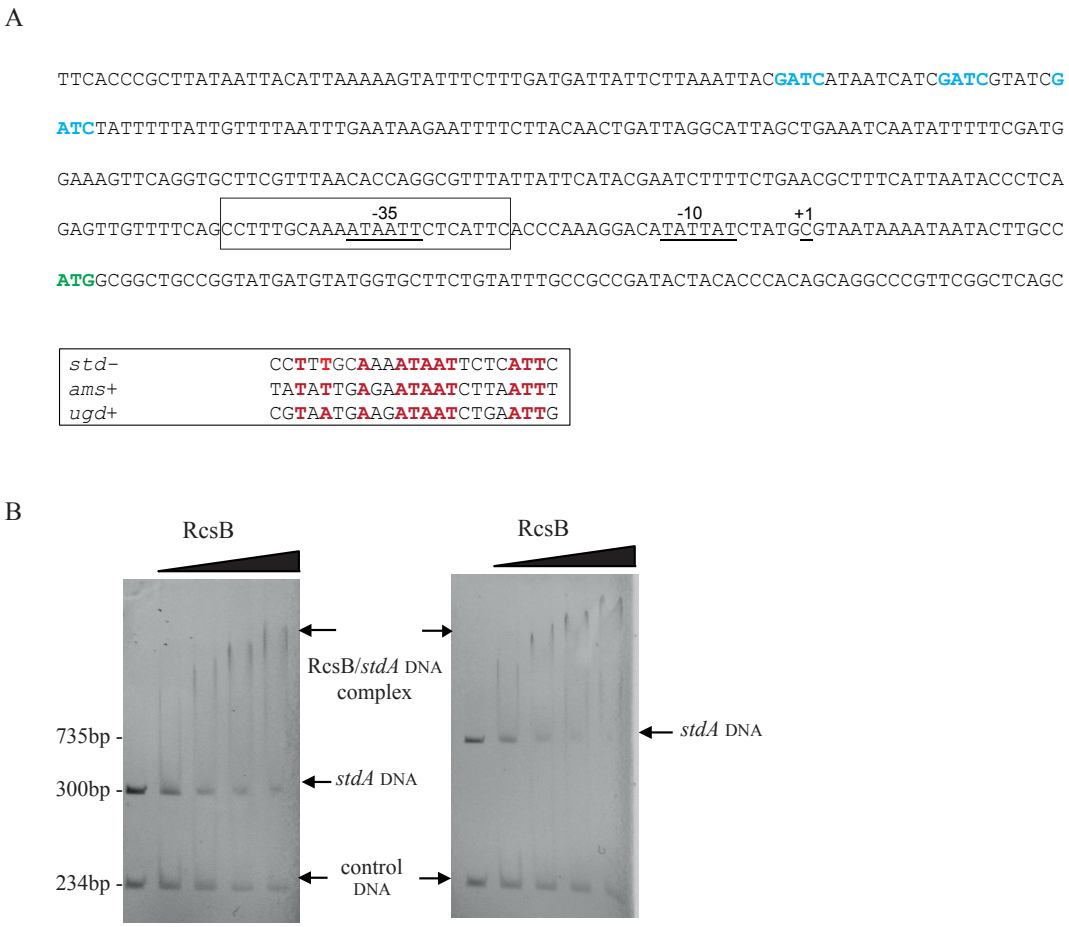

Figure C1. 5 RcsB binds to *std* promoter A. *In silico* analysis showing RcsB putative binding sites in the *std* promoter. In blue are highlighted the three GATC sites in the *std* promoter, in green the start codon of the *stdA* gene, underlined are highlighted the -35, -10 and +1 sites and the RcsB putative binding site is distinct with a box. B. Electrophoretic Mobility Shift Assay (EMSA) of the *std* promoter with increasing concentrations of RcsB protein shows the slow mobility of the RcsB-s*tdA* DNA complex compared with the control DNA.

Expression and autoregulation of the transcription factor HdfR

HdfR is essential for *std* expression in both the wild type (this study) and the Dam$^-$ background (Jakomin et al., 2008). Three GATC sites are present upstream the *std* promoter, in positions -243, -230 and -221 upstream of the transcription start of *std*. The methylation state of GATC sites can be affected by DNA-protein interactions. Therefore, Dam

methylation can act as a regulator of transcription by influencing binding of RNA polymerase or transcriptional factors (Wion & Casadesús, 2006). Previously in the lab, purified HdfR-His_6 was used to study the interaction of HdfR with the *std* promoter region (from -295 to +11) *in vitro*, and to test the effect of Dam methylation on HdfR binding. Unpublished data show that HdfR binds the *std* regulatory region when the three GATCs of this region are non-methylated, while it is unable to bind when the three GATCs are methylated or hemimethylated.

Chromatin Immunoprecipitation (ChIP) coupled with quantitative PCR was used to investigate HdfR-*std* promoter interaction *in vivo* using the strain Δ*dam hdfR*-3xFLAG. A chromatin fragment containing the *std* promoter was 13 fold enriched in the inmunoprecipitated (IP) sample compared with the mock IP sample. Therefore, confirming *in vitro* data, HdfR binds to the non-methylated *std* promoter (**Figure C1. 6**).

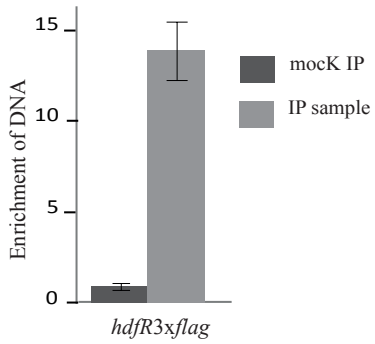

Figure C1. 6 Chromatin Immunoprecipitation (ChIP) coupled with quantitative PCR analysis of HdfR binding to the *std* promoter.

However, and as shown above, *std* expression in wild type cells is only detected in a small subpopulation, whereas Dam^- cells show high level of expression. We wondered if these differences in expression were due to a difference in the level of the expression of *hdfR*. To address this question, the β-galactosidase activity of an *hdfR::lacZ* fusion was measured in the wild type and in a Dam^- strain in exponential and stationary cultures (**Figure C1. 7**). No significant differences were observed in *hdfR* expression, indicating that the differences in *std* expression observed between Dam^- and wild type *Salmonella* cannot be explained by different *hdfR* expression levels.

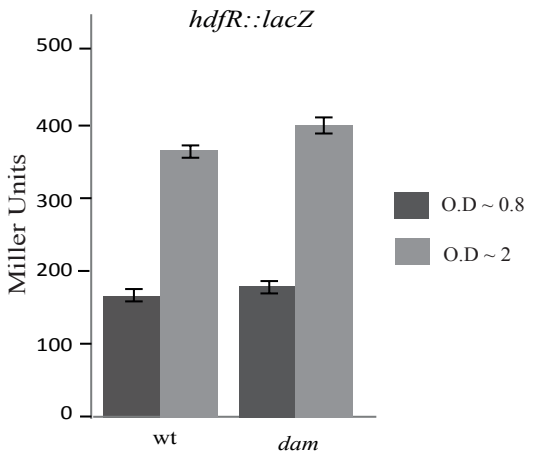

Figure C1. 7 β-galactosidase activity of *hdfR::lacZ* in exponential and stationary phase in a wild type and Dam⁻ backgrounds.

HdfR is a LysR-type transcriptional regulator, and these transcription factors commonly activate the expression of target genes but repress their own expression. To investigate whether HdfR represses its own expression, the intergenic region between *hdfR* and the upstream gene in the chromosome (*yifE*), which most likely contains the *hdfR* promoter, was cloned on the promoter-probe vector pIC552 (Macián et al., 1994), generating a transcriptional fusion with the *lacZ* gene (pIZ2320) (**Figure C1. 8A**). The activity of the p*hdfR*::lacZ fusion was monitored in HdfR$^+$ and HdfR$^-$ backgrounds to determine whether HdfR undergoes autogenous transcriptional regulation.

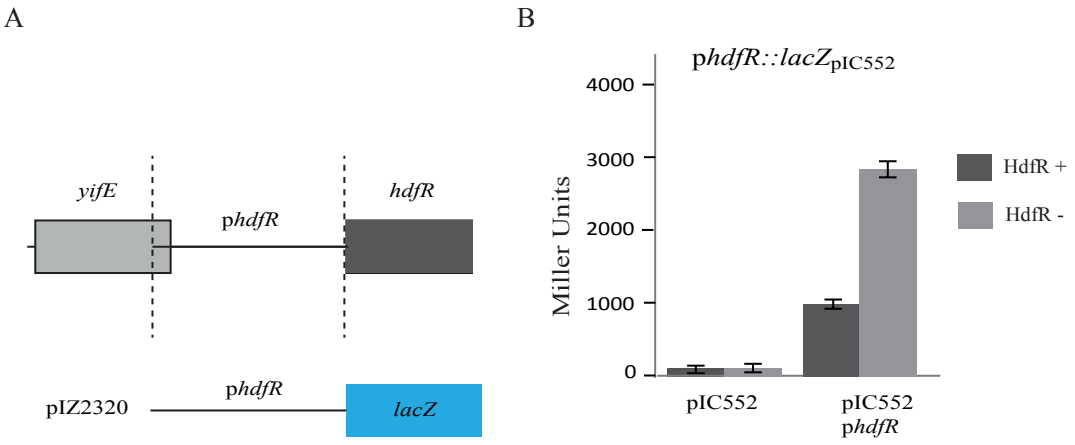

Figure C1. 8 Autoregulation of *hdfR*. A. Diagram of the *hdfR* promoter region. The DNA fragment cloned on pIC552 is represented. The fragment contains the *hdfR* promoter region. **B.** β-Galactosidase activities of pIC552 and pIC552 p*hdfR* in the presence and in the absence of HdfR.

As shown in **Figure C1. 8B**, HdfR undergoes autogenous repression of the *hdfR* promoter, in a fashion analogous to other LysR-type transcription factors. Autogenous repression might ensure a constant intracellular level of the protein. Moreover, as it will be shown in Chapter 2, we have identified additional factors that affect *hdfR* expression.

In order to determine whether high HdfR levels might affect *std* expression, we placed *hdfR* under the control of the heterologous promoter P_{LtetO}. Despite the fact that P_{LtetO} is a moderate promoter, higher *hdfR* expression levels were obtained (**Figure C1. 9A**), and the autorepression was avoided. The expression of the *stdA*::*gfp* and *stdA::lacZ* fusions was monitored by fluorescence analysis and β-galactosidase assays, respectively.

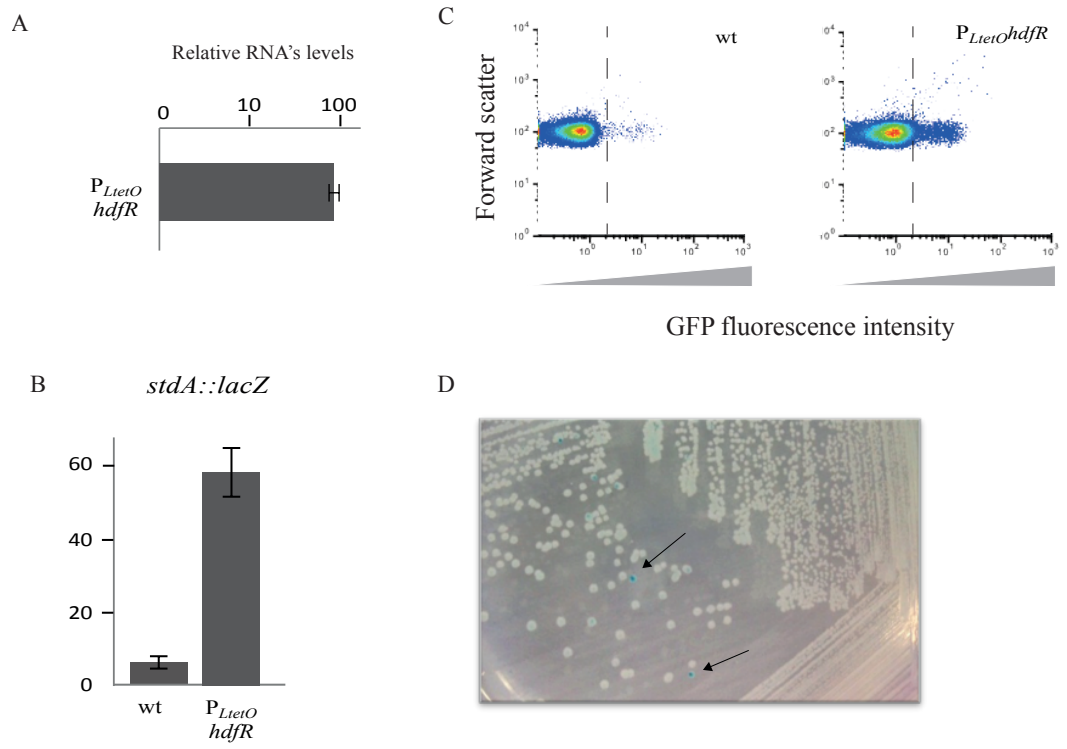

Figure C1. 9 Constitutive expression of *hdfR* increase *std*^{ON} subpopulation. A. Constitutive expression from the heterologous promoter P_{LtetO} increases 100 fold the expression of *hdfR* compared with the wild type strain. Data are normalized to the RNA level obtained in wild type *Salmonella* cells (Value for wild type=1; not shown). **B.** Constitutive expression from the heterologous promoter P_{LtetO} increases 10 fold the expression of the *stdA::lacZ* fusion compared with the wild type strain. **C.** Fluorescence intensity of the *stdA::gfp* fusion in a P_{LtetO}*hdfR* shows an increase of the mean fluorescence and an increment of the *std*^{ON} subpopulation. **C.** *stdA::lacZ* P_{LtetO}*hdfR* on X-gal plates shows colonies with different *stdA::lacZ* expression (blue colonies pointed with arrows).

Increased *hdfR* expression results in increased *stdA* transcription (**Figure C1. 9B**), and in an enrichment of the Std^{ON} subpopulation (**Figure C1. 9C**). The latter observation was made not only by flow cytometry but also on X-gal plates, where two types of colonies were distinguished (**Figure C1. 9D**). In this case the heterogeneity of the population cannot be due to different levels of HdfR in the cells since expression is driven from a constitutive promoter. Therefore another factor must govern variability in the expression of *std.*

Although several repressors of *std* expression have been described in previous studies and also in this work, the mechanisms that permit *std* expression inside the animal intestine were unknown. To search for factors that might activate expression of the *std* operon in a wild type (Dam^+) background, a genetic screen for activators of the *std* operon was performed using a pBR328-based multicopy plasmid library of the *Salmonella enterica* genome (López-Garrido & Casadesús, 2012). A Dam^+ strain carrying a *stdA*::*lacZ* translational fusion (SV5182; (Jakomin et al., 2008)), was used as reporter for the screen. This strain does not show *lacZ* expression at all, since transcription is driven by the *std* promoter which is strongly repressed under laboratory conditions. Strain SV5182 was transduced with 9 pools of the plasmid library, each containing around 1,000 independent clones. Transductants were selected on LB plates containing chloramphenicol and X-gal. Forty-five colonies with increased β-galactosidase activity (blue colonies) were chosen, and 22 independent candidates that retained high β-galactosidase activity after re-transformation of their plasmid were further analyzed. The DNA fragments contained in the plasmid of 15 candidates were sequenced using specific primers flanking the insertion site. Surprisingly, DNA sequencing revealed that 14 candidates contained the *std* fimbrial gene cluster (pBR328*std,* annotated as pIZ2318), among other neighboring genes, and one candidate contained the *std* fimbrial operon except *stdF* (pBR328*std*Δ*stdF*, annotated as pIZ2319). These results indicate that activators of the *std* operon were contained inside the operon itself; in other words, they suggest self-regulation of the operon. Furthermore, they revealed that the StdF might play a role in autogenous activation since *stdA::lacZ* expression decreased considerably when the downstream gene *stdF* was not present. Expression is dependent on HdfR, highlighting again the major role of this protein in *std* expression (**Figure C1. 10**).

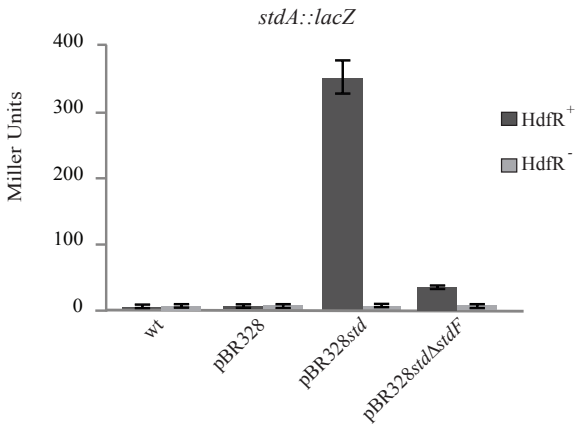

Figure C1. 10 Autoregulation of the *std* operon. β-galactosidase activity of a *stdA::lacZ* fusion in the presence of plasmid containing the *std* operon, in $HdfR^+$ and $HdfR^-$ genetic backgrounds.

Interestingly, previous results from our lab had identified the gene products of *stdE* and *stdF* as repressors of SPI-1 expression (López-Garrido & Casadesús, 2012). These results, together with the fact that in other fimbrial operons both structural and regulatory elements are encoded in the same operon, fueled further studies on the involvement of StdE and StdF in self-regulation of *std* expression. The strain carrying the *stdA::gfp* fusion was used to test the effect of StdE and StdF on expression of the *std* operon. For this purpose, we placed the heterologous promoter P_{LtetO} (Lutz & Bujard, 1997; McQuiston et al., 2008) upstream of *stdEF* (P_{LtetO}*stdEF*) to ensure constitutive expression on both genes. (**Figure C1. 11A**). Expression of *stdA* was monitored by flow cytometry. As observed in **Figure C1. 11B**, deletion of *stdEF,* $P_{LtetO}\Delta$*stdEF,* results in loss of the cell subpopulation that expresses *gfp*. In turn, constitutive expression of *stdEF* slightly increases *gfp* mean fluorescence and significantly increases the size of the std^{ON} subpopulation (from 0,3% to 3%). Interestingly, in a Dam^- background, deletion of *stdEF* causes a decrease in *gfp* expression, whereas constitutive expression increases *gfp* expression and results in an altered pattern of expression in the cell population (higher heterogeneity) (**Figure C1. 11B**). The same pattern was observed using the *stdA::lacZ* fusion in a P_{LtetO}*stdEF* background on X-gal plates: an increment of blue colonies was observed, with some of them showing a darker blue (**Figure C1. 11C**).

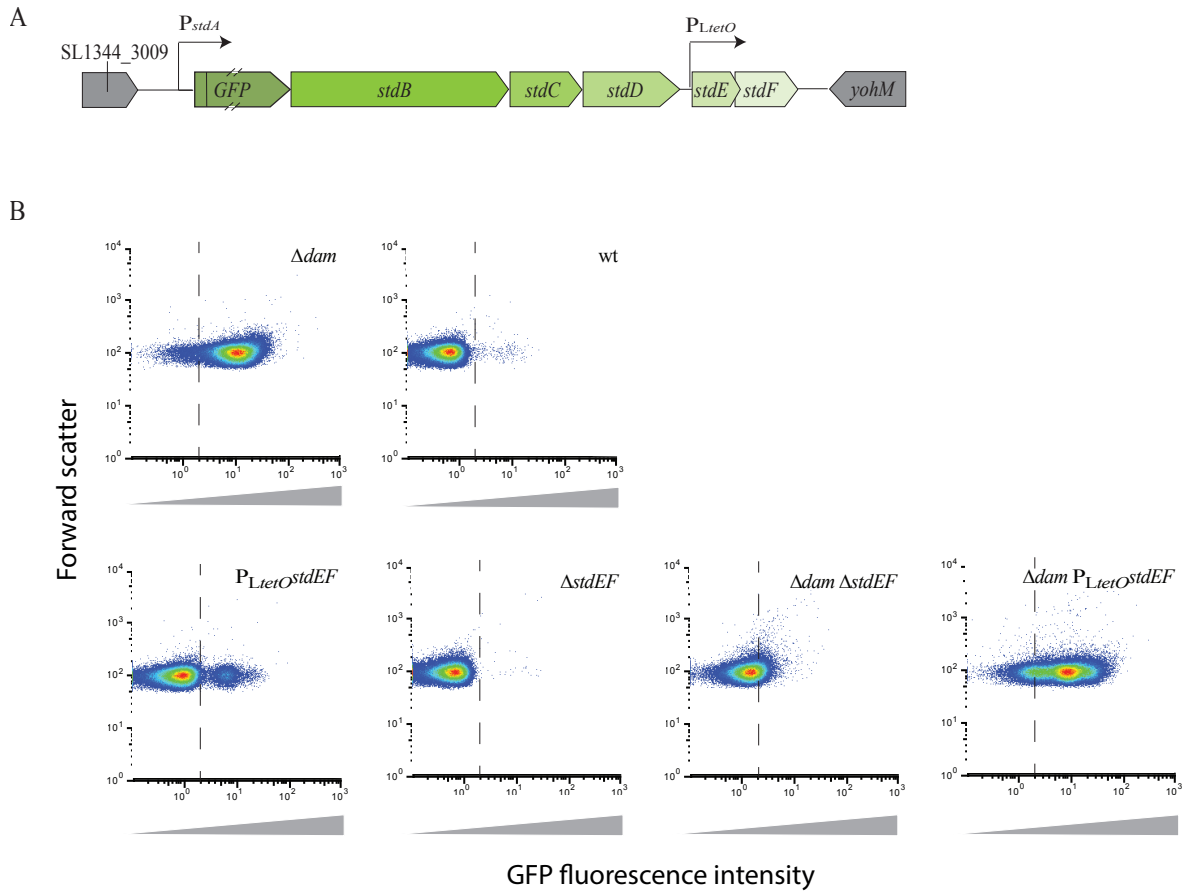

Figure C1. 11 Effect of constitutive expression of *stdEF* on *stdA*::*gfp* expression. A. Diagram showing the construction used to monitor *stdA::gfp* fusion under *stdEF* constitutive expression. **B.** Single cell analysis of *stdA::gfp*-expressing cells in different genetic backgrounds. **C.** P_{LtetO}*stdEF stdA::lacZ* on X-gal plates with colonies showing and increase of *stdA::lacZ* basal expression and some colonies with higher *stdA::lacZ* expression (darker blue colonies pointed with arrows).

These results (**Figure C1. 11**) confirm that StdE and StdF have a role in the expression of the *std* operon, not only in the wild type strain but also in the Dam^{-} mutant background.

To determine if StdE and StdF were able to bind the *std* promoter region, we used Chromatin Immunoprecipitation (ChIP) coupled with quantitative PCR to investigate a putative StdE/StdF-*std* promoter interaction *in vivo*. The strains used were Δ*dam stdE*-3xFLAG and

Δ*dam stdF*-3xFLAG, since the Dam⁻ background ensures a higher expression level of *stdE* and *stdF*.

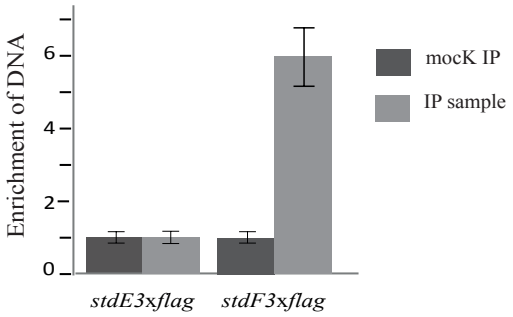

Figure C1. 12 Chromatin Immunoprecipitation (ChIP) coupled with quantitative PCR analysis of binding to the *std* promoter. ChIP-qPCR performed with Δ*dam stdE*-3xFLAG and Δ*dam stdF*-3xFLAG.

According to the results presented in **Figure C1. 12**, the chromatin fragment containing the *std* promoter immunoprecipitated with StdF-3xFLAG is enriched 6 times compared with the mock immunoprecipitated sample, indicating that StdF binds to the *std* promoter *in vivo* and suggesting a pivotal role for StdF in *std* transcription. However, no binding was observed for StdE, which might suggest that this protein is not relevant for expression of the *std* operon or that it plays an indirect role. Note that ChIP-qPCR assay do not allow to distinguish between direct or indirect binding to the DNA sequence. On the other hand, these data corroborate the major role of StdF unveiled by the genetic screen described above (in which a candidate lacking the downstream gene of the operon showed a decreased in *stdA::lacZ* expression).

Figure C1. 13 shows a diagram of the players identified in the *std* regulation. The diagram simulates the *std* operon in the ON state. As it was already known, under these circumstances, the Dam methylase does not act over the GATC sites of the *std* promoter; therefore the GATCs are non-methylated and HdfR binds the *std* regulatory region. In turn, *stdF* expression, as described in this Thesis, supports *std* expression through an autoregulatory positive loop and StdF binds the *std* promoter region in a Dam-independent manner. In addition, wild type levels of RcsB do not bind the *std* promoter, but high levels of RcsB do bind and as shown in **Figure C1. 5** repress *std* expression in Dam⁻ and wild type backgrounds.

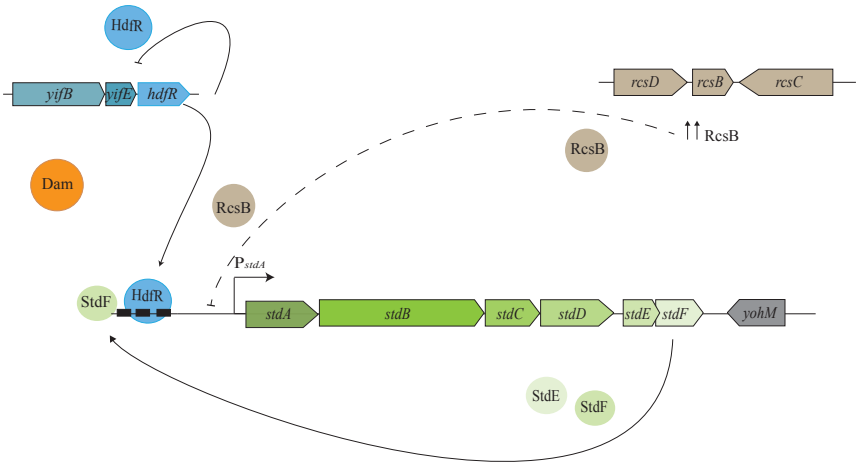

Figure C1. 13 Regulation of the *std* operon by StdF, HdfR and RcsB. Positive effects are indicated by lines ending in arrowheads, whereas blunt lines indicate negative regulatory effects. Dashed lines indicate regulation that do not apply under the diagram circumstances. The three black squares represent the three GATC sites in the regulatory region of the *std* operon, that in the circumstances detailed above would be non-mehylated.

Methylation state of the *std* operon

Expression of *std* is detected in a Dam$^-$ background, whereas in a wild type strain expression is strongly repressed (Balbontín et al., 2006; Jakomin et al., 2008). Only a minor subpopulation expresses the *std* operon, according to single cell analysis described above. The *std* promoter region contains three GATC sites in positions -243, -230 and -221 upstream of the start of *std* transcription. Experiments carried out in our laboratory have shown that the transcription factor HdfR, needed for *std* expression, binds to the *std* promoter region when the GATC sites are non-methylated. HdfR is unable to bind either methylated nor hemimethylated DNA (unpublished results). These observations raise the possibility that the DNA methylation pattern of the *std* upstream activating sequence (UAS) might be different in Std^{ON} and *Std*^{OFF} subpopulations. If this view is correct, only a small number of cells might harbor the DNA methylation pattern that allows HdfR binding and, as a consequence, *std* expression. The hypothesis of a different methylation pattern within the population is supported by the partial loss of Std^{ON} subpopulation upon overproduction of Dam methylase (cloned on pTP166) (**Figure C1. 14**).

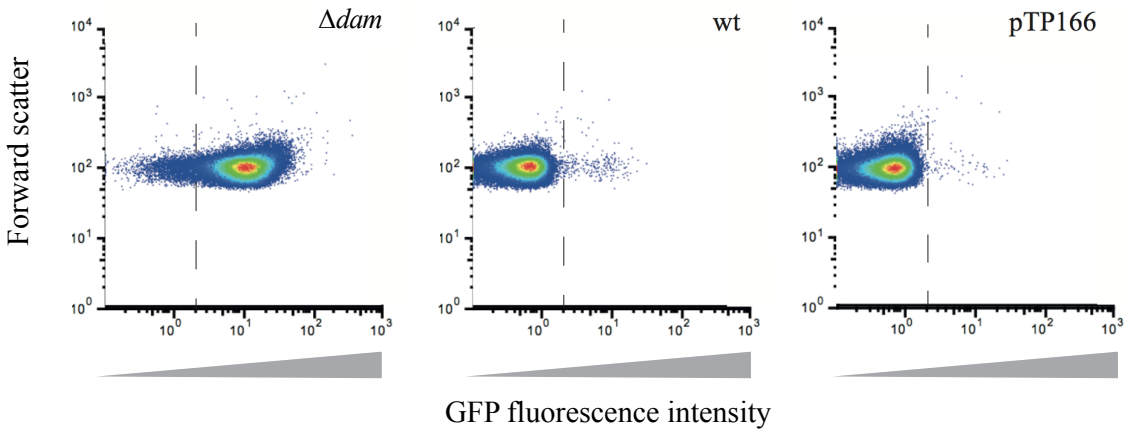

Figure C1. 14 Regulation of *std* expression and formation of *std* subpopulations by Dam methylation. *stdA::gfp* fluorescence intensity in Δ*dam,* wild type and in a strain that overproduces Dam methylase (pTP166).

To identify the methylation state of the *std* promoter *in vivo*, Southern blot experiments were performed. The methylation state of individual GATC sites was inferred from restriction analysis using enzymes that cut GATC sequences depending on their methylation state (MboI- non methylated, DpnI- methylated, and Sau3AI- methylated and non methylated). A Dam$^-$ mutant was used as a control (**Figure C1. 15A**)

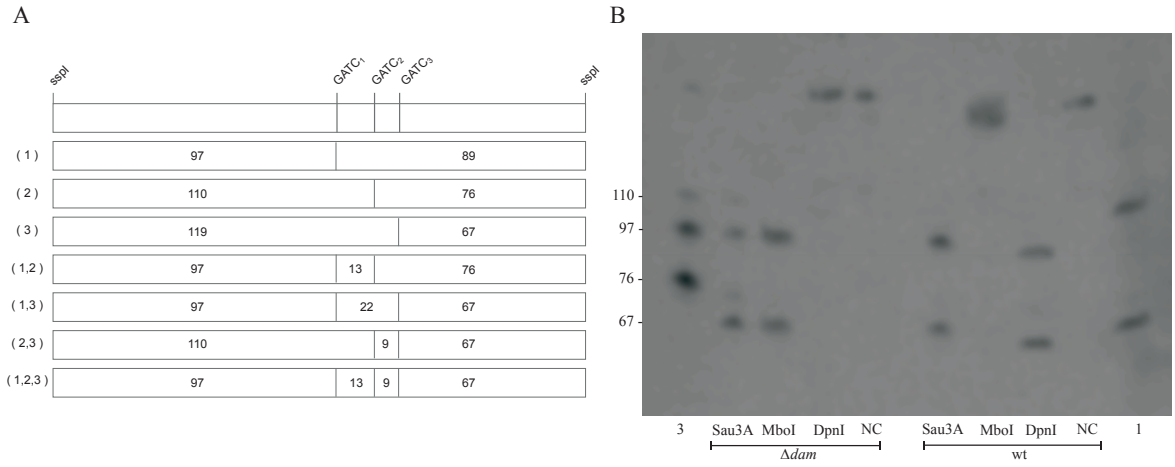

Figure C1. 15 Methylation state of GATC sites in the *std* regulatory region in wild type and Dam$^-$ backgrounds. A. Diagram of the SspI-SspI fragment and patterns of fragments obtained after cleavage of the different GATC sites. Sample 3 refers to a wild type strain with the $GATC_3$ mutated and treated with Sau3AI, that results in 97, 76 and 13 bp fragments. Another control, sample 1, refers to a wild type strain with the $GATC_1$ mutated and treated with Sau3AI, that results in 110, 67 and 9 bp fragments. **B.** Southern blot of genomic DNA obtained from wild type and Dam$^-$ cultures and digested with SspI (control, NC) and DpnI, MboI or Sau3AI.

As shown in **Figure C1. 15B**, the three GATC sites ($GATC^1$, $GATC^2$, and $GATC^3$) are fully methylated in a wild type background. Considering that the majority of the population is in an Std^{OFF} state, in the Southern the wild type strain largely corresponds to the Std^{OFF} subpopulation.

These results correlate well with previous assays of HdfR binding to the *std* promoter, in which binding occurred exclusively to non-methylated DNA. Identification of the DNA methylation pattern of the Std^{ON} subpopulation was difficult due to its small size (only 0,3 to 3%) of the population.

StdE and StdF are located in the cytoplasm of *Salmonella enterica*

StdE and StdF are homologues of known transcriptional regulators. Specifically, StdE shares 40-50% identity with the transcriptional activators GrlA from *E.coli* and CaiF from *Enterobacter cloacae*. In turn, StdF is related to the SPI-1 *Salmonella* protein SprB, a transcriptional regulator that represses the *hilD* promoter and activates the *siiA* promoter (López-Garrido & Casadesús, 2012). This homology with transcriptional regulators correlates well with the data presented in this chapter indicating that these proteins exert autogenous regulation of *std* transcription and also control *hdfR* expression (see above).

The subcellular location of StdE and StdF was studied using 3xFLAG-tagged versions of the proteins in a Dam^- background. Electrophoretic separation of cell fractions (cytoplasm, inner membrane and outer membrane) was performed, followed by Western blot analysis of the separated protein samples using a commercial anti-FLAG antibody. Based on the results obtained, we can conclude that both StdE and StdF are cytoplasmic proteins in *Salmonella enterica* (**Figure C1. 16**).

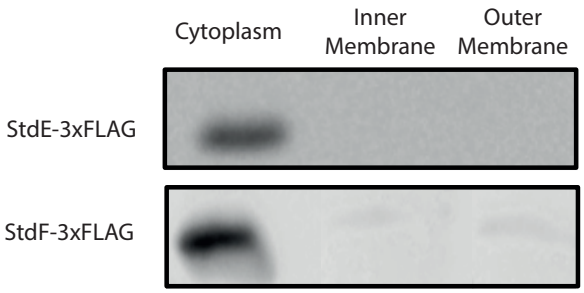

Figure C1. 16 Subcellular localization of StdE and StdF proteins in *S. enterica* subcellular fractions. 3XFLAG versions of the proteins were localized with anti-FLAG Western hybridization for different subcellular fractions (cytoplasm, inner membrane and outer membrane).

Search for an internal promoter in the *std* operon

Because a fraction of the bacterial population expresses the *std* operon under conditions in which the main promoter appears to be inactive, we wondered whether expression of the StdE and StdF activators might occur independently of the *std* main promoter. Previous results in the lab had shown that the *stdE* and *stdF* genes were transcribed from the *std* promoter (upstream of *stdA*) in a Dam$^-$ background (López-Garrido & Casadesús, 2012). However, the same study raised the possibility that an internal promoter might exist that allow transcription of these two genes independently. To test this possibility we constructed a *stdE::gfp* fusion, in which the upstream genes (up to *stdD*) were deleted (together with the main promoter) (**Figure C1. 17A)**. As observed in **Figure C1. 17 B**, low expression is observed when cells are run in a flow cytometer. We are confident that this expression, no matter if very low, is real, since it was repeatedly observed for 10 biological replicates run in different days, and previous studies had reported an expression of *stdE* that was not detected for the remaining *std* genes (López-Garrido & Casadesús, 2012). This result suggests the presence of a promoter that may drive the expression of *stdE,* and probably also of *stdF*.

Several attempts have been done to identify this putative internal promoter. Cloning of different gene fragments onto the promoter-probe plasmid pIC552 did not reveal promoter activity. 5´RACE experiments performed in wild type and Dam$^-$ genetic backgrounds failed also in the identification of such promoter. We are currently working an alternative solution for this identification since we do think that an *std* internal promoter exists.

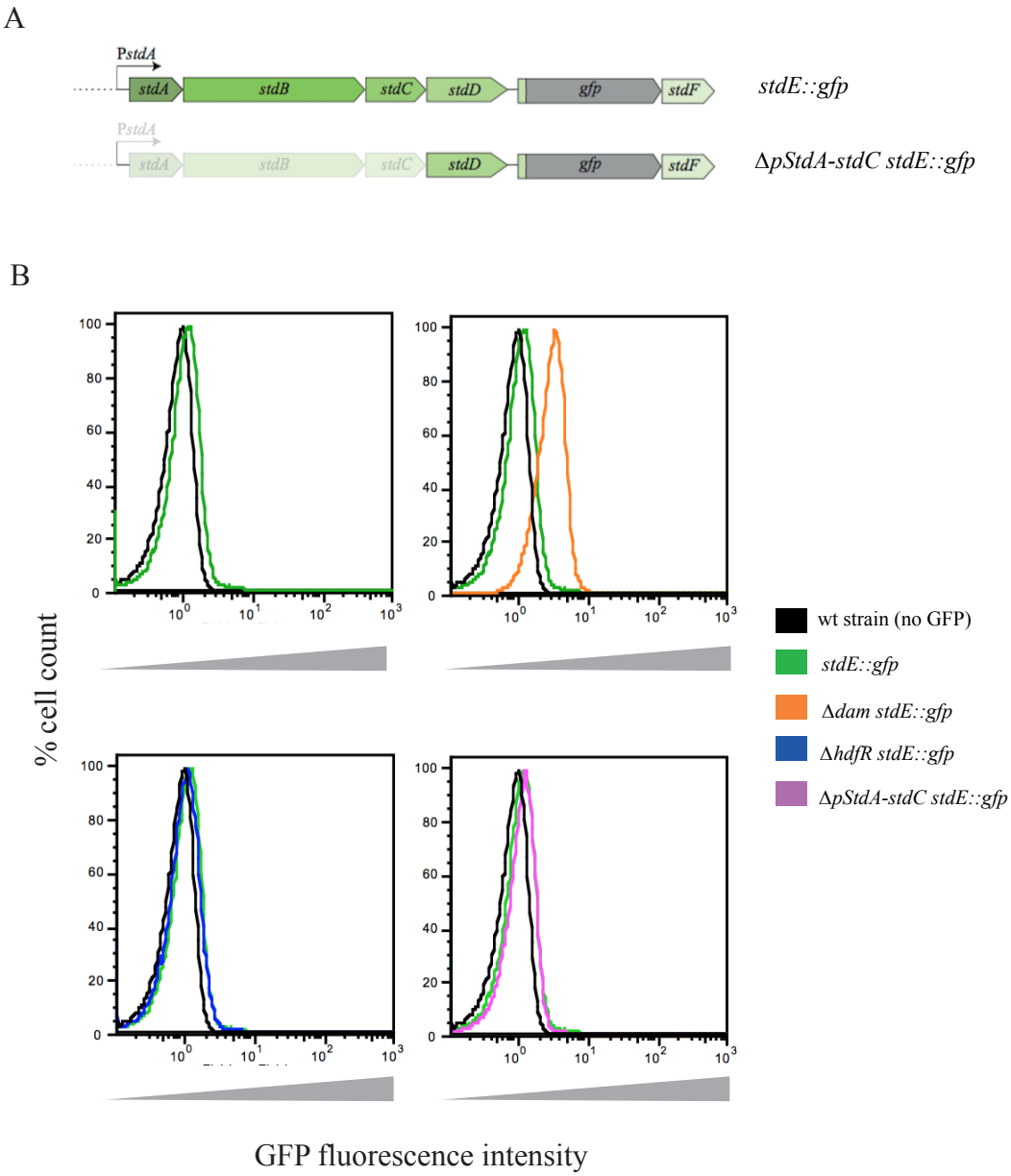

Figure C1. 17 Evidence of the existence of an internal promoter upstream *stdE*. **A.** Diagram of the Δ*pStdA-stdC stdE::gfp* construction. **B.** *stdE::gfp* expression in different genetic backgrounds. Dam⁻ and HdfR⁻ backgrounds are used to define *stdE*::*gfp* expression.

Chapter II: Impact of *stdE* and *stdF* overexpression in *Salmonella enterica*

Transcriptomic analysis of *S. enterica* cells constitutively expressing *stdEF*

In order to identify genes regulated by *stdE* and *stdF*, comparative transcriptomic analyses were performed in a strain that constitutively expressed *stdEF* and in a control strain carrying an in-frame deletion of both genes. To achieve moderate, constitutive expression of *stdE* and *stdF*, strain SV8141 was used (P_{LtetO}*stdEF*). In this strain, transcription of both genes is driven by the P_{LtetO} promoter (Lutz & Bujard, 1997; McQuiston et al., 2008). To avoid potential artefacts, the native *stdA* promoter and all the genes upstream of *stdE* in the *std* operon (**Figure C2. 1A**) were deleted, and the P_{LtetO} promoter was placed upstream of *stdE* in the chromosome. The control strain contained the same insertion of the P_{LtetO} promoter in the chromosome, lacking the upper part of the *std* operon but carrying also a deletion of *stdE* and *stdF* (SV8142; P_{LtetO}Δ*stdEF*, (**Figure C2. 1B**).

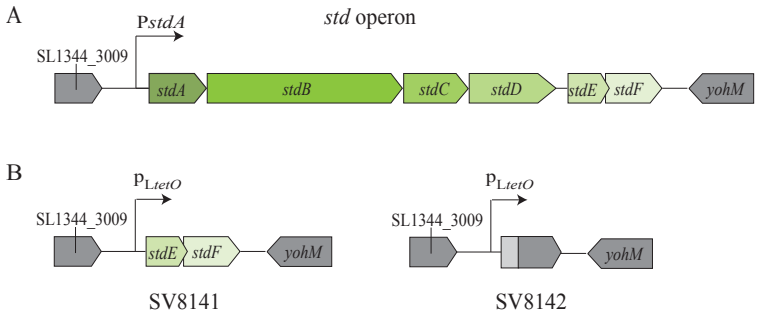

Figure C2. 1. Diagrams of the *std* operon and of the strains SV8141 and SV8142. **A.** The *std* operon and surrounding genes in a *Salmonella enterica* wild type strain. Transcription of the operon is driven by the *stdA* promoter (P_{stdA}). **B.** Diagrams of the *std* operon and surrounding genes in the strains used for transcriptomic analysis (P_{LtetO}*stdEF;* SV8141 and P_{LtetO}Δ*stdEF;* SV8142) is shown.

Transcriptomic analysis was performed using an array custom prepared by Dr. A. Juárez and co-workers (Paytubi et al., 2014), with RNA isolated from cells grown to stationary phase (OD_{600}~2). Raw data from transcriptomic analysis have been deposited at the Gene Expression Omnibus (G.E.O) database (Edgar et al., 2002), with accession number GSE45488.

The results show that, under the conditions used in the study, a large number of *Salmonella* loci showed differences in their RNA levels in an StdEF-dependent manner. Relevant data are summarized in **Figure C2. 2** in which genes showing a higher than 4-fold difference in

their RNA amount are depicted. Complete data from the transcriptomic analysis, along with gene descriptions and fold changes, are shown in **Supplementary Table SM.1**.

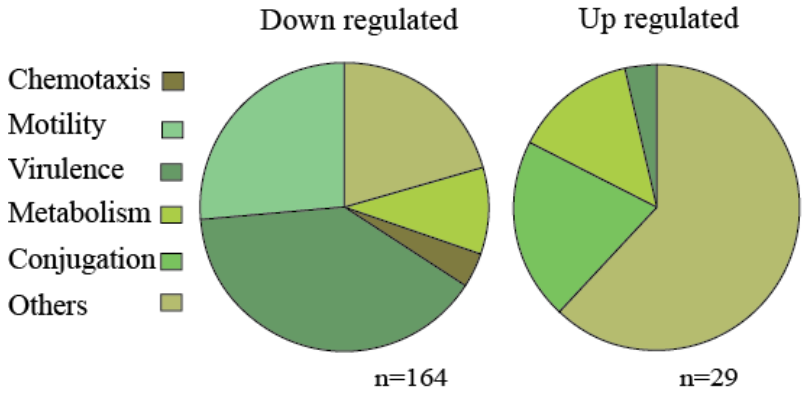

Figure C2. 2 Pie chart summarizing the microarray data obtained. Numbers of genes up- and down-regulated, and physiological processes in which they are involved.

As shown in **Figure C2. 2**, almost 200 genes showed a difference in expression higher than 4 fold upon constitutive expression of *stdEF*. About 85% of these genes appear to be down-regulated by StdEF, suggesting that these proteins may be often repressors of gene expression in *Salmonella.* Among the down-regulated loci, genes involved in virulence and motility are highly represented. Regarding virulence, genes belonging to pathogenicity islands SPI-1 and SPI-4 are repressed by StdEF. As mentioned before, motility related genes are also found among down-regulated genes. The list includes not only *flhDC*, which encodes the master regulator of flagellar synthesis (Kutsukake et al., 1990), but also the *flg*, *flh*, *fli,* and *motAB* operons (**Supplementary Table SM.1**) are also repressed. FlhDC has been described as an activator of SPI-1 genes (Chubiz et al., 2010; Saini et al., 2008), which might explain the presence of strongly down-regulated SPI-1 genes in our transcriptomic analysis and in a previous study focused on the involvement of StdEF in *Salmonella* virulence (López-Garrido & Casadesús, 2012). Among down-regulated genes, we also find *ygiD*, annotated as a hypothetical protein, which seems to be involved in biofilm formation (**Supplementary Table SM.1**). The *che* operon and the *trg* and *aer* genes involved in chemotaxis were also found to be down-regulated (**Figure C2.2**).

Among the up-regulated loci, we observe a high representation of genes involved in general metabolism, along with genes of the *tra* operon encoded on the pSLT plasmid (**Supplementary Table SM.1)**. The only up-regulated gene that may be involved in

virulence is the SL0982 locus, annotated as a "Gifsy-2 prophage attachment and invasion protein homolog", which shows a 64-fold increase compared with the control strain. Interestingly, we also found *yifA* among the up-regulated genes, with an 8.4-fold change of expression. *yifA* (henceforth, *hdfR)* encodes the transcriptional activator HdfR, which is responsible for the expression of the *std* operon not only in a Dam$^-$ mutant (Jakomin et al., 2008) but also in a wild type background, as it was shown in Chapter 1. Regulation of *hdfR* by StdEF defines a positive feedback loop for autogenous control of the *std* operon. Additional results regarding mechanisms of self-regulation of the *std* operon were presented in Chapter 1.

Validation of transcriptomic data

To validate the transcriptomic data described above, we performed quantitative RT-PCR analysis of some of the genes differentially expressed in the presence of StdEF. We analysed expression of genes involved in motility (*motA, flgE*), conjugation (*traA*), chemotaxis (*trg*), virulence (*hilA*, *sipB*) and the transcriptional regulator of the *std* operon, *hdfR*. Data presented in **Figure C2. 3** show that all the genes analysed undergo a change in expression in cells that constitutively express *stdEF* (P_{LtetO}*stdEF*), compared with the control ($P_{LtetO}\Delta$*stdEF*) and with wild type *S. enterica*. Changes in gene expression correlate well with those observed in the array, with fold-changes higher than 10-fold in all cases.

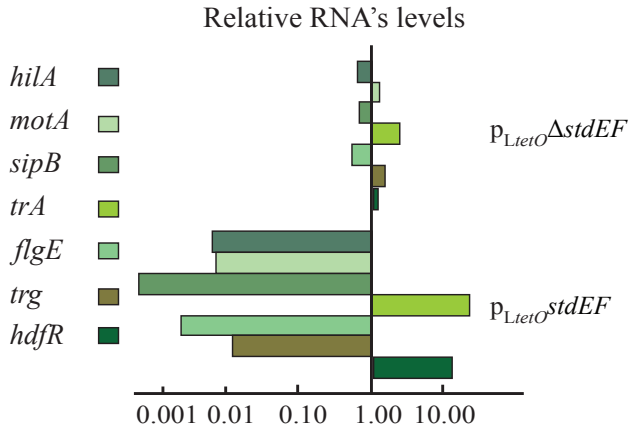

Figure C2. 3 Validation of microarray data. RNA levels for some of the genes under *stdEF* control identified by microarray data in strains P_{LtetO}*stdEF* and $P_{LtetO\Delta}$*stdEF*. Data are normalized to the RNA level obtained in wild type *Salmonella* cells (Value for wild type=1; not shown). RNA levels were determined for at least 3 biological replicates and a representative experiment is shown.

Although, as shown in chapter 1, the *std* operon is tightly repressed under the conditions employed in our microarray analysis (lab conditions), the expression levels of the analysed genes in the P_{LtetO}Δ*stdEF* strain vary slightly from the normalized *Salmonella* wild type strain. The existence of a small fraction of the population that expresses *std* (Std^{ON} cells) may explain this tiny difference, reinforcing the view that *std* is expressed under laboratory conditions in a subpopulation of bacterial cells.

Functional validation of the transcriptomic data was also performed. In the case of *Salmonella* pathogenicity island-1 (SPI-1), regulation by StdEF had been previously proven in a study from our laboratory (López-Garrido & Casadesús, 2012). Constitutive expression of *stdE* and *stdF* had been shown to down-regulate SPI-1 genes, to decrease invasion of HeLa cells *in vitro*, and to attenuate *Salmonella* virulence upon infection of BALB/c mice (López-Garrido & Casadesús, 2012). The results presented in this Thesis correlate well with such data since a large number of SPI-1 genes appear to be down-regulated in the transcriptomic analysis. As a further functional validation of the transcriptomic analysis, we carried out tests of motility, biofilm formation, and conjugation in *Salmonella* strains that constitutively expressed the *stdE* and *stdF* genes **(Figure C2. 4)**.

The three phenotypes under examination respond to *stdEF* expression. Regarding motility, *Salmonella* cells expressing *stdEF* show no motility on a soft agar plate, indicating that constitutive expression of *stdEF* impairs motility of *S. enterica* cells. This phenotype correlates well with the transcriptomic data that show down-regulation of flagellar genes. Interestingly, the control strain (P_{LtetO}Δ*stdEF*) is more motile than *Salmonella* wild type cells (**Figure C2. 4A**). Control cells are wild-type cells in which the *std* operon has been deleted, as described in Materials and Methods and shown in **Figure C2. 1B**. This result confirms a role of the *std* operon in repression of motility, and reinforce the view that the *std* operon is expressed in a small fraction of the population (0.3-3%), under laboratory conditions.

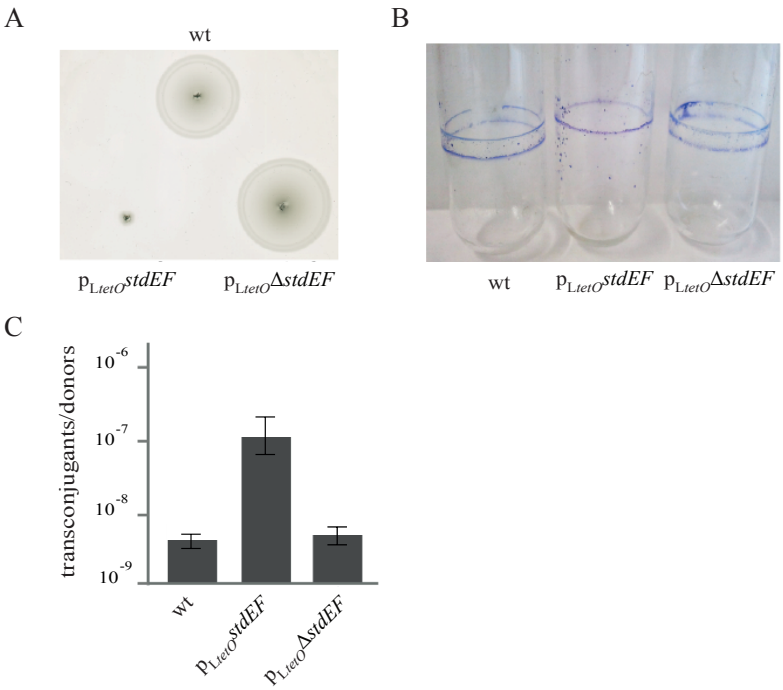

Figure C2. 4 Functional validation of the transcriptomic analysis. A. Motility agar plates (see Materials and Methods) were used to test the motility of the strains used in this study. The experiments were performed in triplicate, and a representative plate is shown. **B.** The biofilm formed by *S. enterica* SL1344 (wt) and the strains P_{LtetO}-*stdEF* and P_{LtetO}-Δ*stdEF* was stained with crystal violet as described in Materials and Methods. The experiments were performed in triplicate, and a representative assay is shown. **C.** Effect of *stdEF* on conjugal transfer of pSLT in microaerobiosis. The recipient was SV4938 in all matings. Donors were wild type *Salmonella* SL1344, and strains P_{LtetO}-*stdEF* and P_{LtetO}-Δ*stdEF*. Data are averages and standard deviations from 3 independent matings.

Balbontín et al. described that *Salmonella* Dam⁻ mutants have a defect in motility (Balbontín et al., 2006). Since *std* overexpression impairs motility in *S. enterica* cells and *std* expression occurs in a Dam⁻ mutants, we cannot rule out the possibility that Dam-dependent regulation of *Salmonella* motility is mediated by *std* expression. To corroborate this possibility we determined the motility of a Dam⁻ mutant lacking *stdEF*.

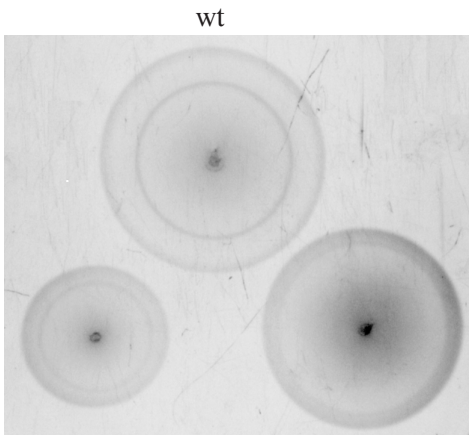

Figure C2. 5 Dam-dependent regulation of *Salmonella* motility is partially mediated by *std* expression. Motility agar plates (see Materials and Methods) were used to test the motility of the strains SL1344, Δ*dam* and Δ*dam* Δ*stdEF* used in this study. The experiments were performed in triplicate, and a representative plate is shown.

As shown in **Figure C2. 5**, the defect in motility observed in a Dam$^-$ mutant is, at least, partially due to the expression of *stdEF*, since the motility defect in Δ*dam Salmonella* is partially restored in the absence of StdEF.

Regarding biofilm formation, we used crystal violet staining to analyse the ability of the P_{LtetO}*stdEF* strain to form biofilm. As observed in **Figure C2. 4B**, biofilm formation decreases in cells that express *stdEF* (P_{LtetO}*stdEF*), compared with the wild type and with a P_{LtetO}Δ*stdEF* strain. In the transcriptomic analysis, the gene *ygiD* appears to be down-regulated. This gene has been annotated as a putative biofilm formation locus in *Salmonella enterica* like its *Escherichia coli* counterpart (Groisman & Ochman 1997; Kelly et al. 2009). To confirm the involvement of *ygiD* in biofilm formation, we analysed the capacity of an YgiD$^-$ null mutant to form biofilm. However, the amount of biofilm formed by the mutant was similar or identical to that of the wild-type strain. This observation indicates that reduced biofilm formation upon *stdEF* overexpression is not due to *ygiD* repression only, and that more genes that we have not been identified are likely to be involved in the process (**Figure C2. 6**).

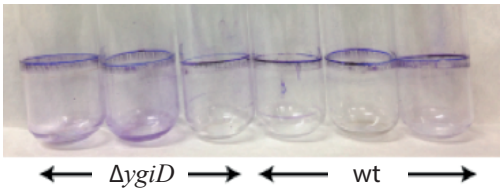

Figure C2. 6 Biofilm formation by *S. enterica* SL1344 cells (wt), and by the Δ*ygiD* strain, detected with crystal violet staining. Three replicates are shown.

The performance of the P_{LtetO}*stdEF* strain in conjugation was also tested (**Figure C2. 4C**). When this strain was used as donor, the frequency of pSLT transfer increased 1.5 orders of magnitude. This increase in conjugation ability is probably due to an increase in the expression of the *tra* operon upon constitutive synthesis of StdEF (**Supplementary Table SM.1**). These results were further confirmed by determination of the β-galactosidase activity of a *traB*::*lacZ* transcriptional fusion in a *Salmonella* P_{LtetO}*stdEF* strain, in a P_{LtetO}Δ*stdEF* strain, and in the wild type. A four-fold increase in *traB::lacZ* activity was observed upon *stdEF* expression (**Figure C2. 7A**).

It has been described that Dam methylation represses *tra* operon expression by maintaining high levels of FinP RNA in the cell. FinP is an antisense RNA whose target is the *traJ* messenger RNA, while TraJ is an activator of the *tra* operon expression. Under these premises, and since a Dam^- mutant has high levels of StdEF, we considered the possibility that Dam-dependent regulation of the *tra* operon might be mediated by StdEF, in a way similar to that described for SPI-1 (López-Garrido & Casadesús, 2012).

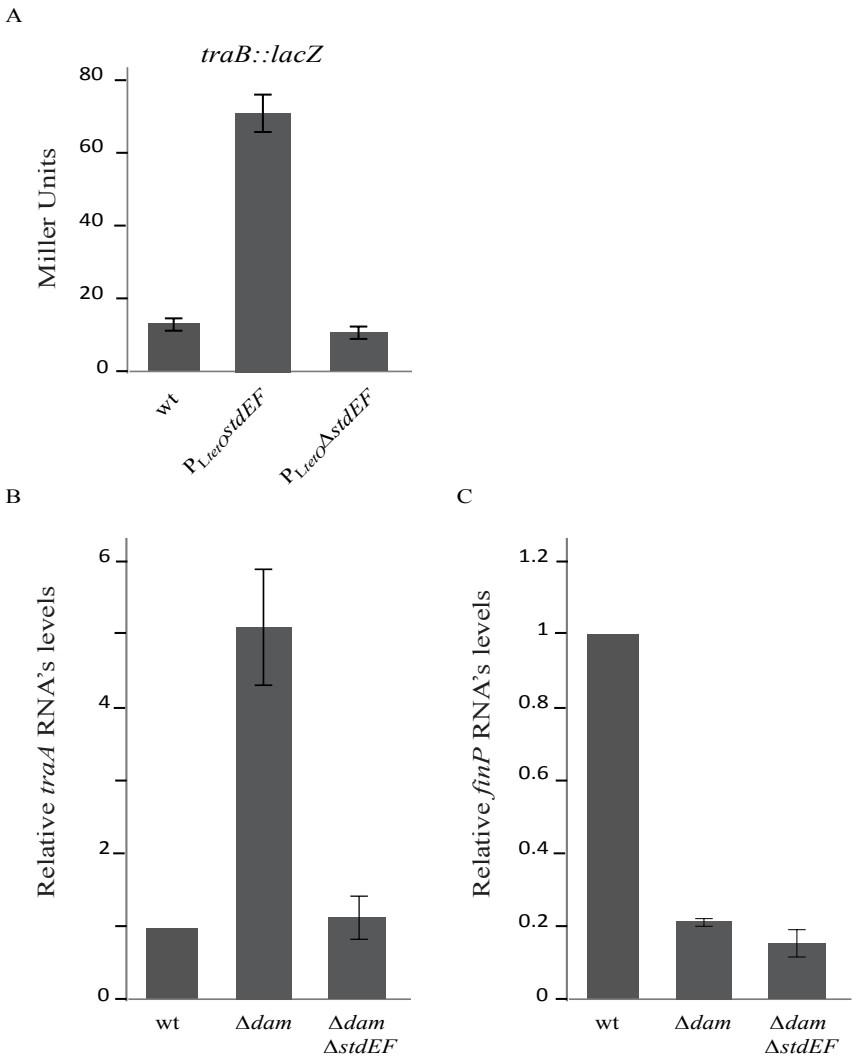

Figure C2. 7 StdEF controls *traB lacZ* expression and mediates Dam-dependent regulation of *tra* operon but not *finP* expression. A. β-galactosidase activity of a *traB::lacZ* fusion. **B.** RNA levels of *traA* in wt, *dam* and *dam* Δ*stdEF* background . **C.** RNA levels of *finP* in wt, Δ*dam* and Δ*dam* Δ*stdEF* background. Data are normalized to the RNA level obtained in wild type *Salmonella* (Value for wild type=1).

Figure C2. 7B shows that upregulation of *traA* expression in a Dam$^-$ background is supressed in the strain lacking *stdEF* (Δ*dam* Δ*stdEF*), suggesting that StdE, StdF or both are necessary for *tra* operon upregulation in Dam mutants. However, *finP* repression remains Dam-dependent in the strain lacking *stdEF*, indicating that these genes are not required for the Dam-dependent control of *finP* (**Figure C2. 7B**).

StdEF global regulation is independent of the transcription factor HdfR

Based on the transcriptomic results obtained, we could not rule out the possibility that the global regulation observed might be exerted through one or more intermediate regulators. One potential regulator was HdfR, whose locus appeared up-regulated in the transcriptomic data. HdfR is a LysR-type transcriptional regulator known to be essential for *std* transcription. Other LysR-type proteins are known to be global transcriptional regulators, as OxyR or LeuO (Broadbent et al., 2010; Haagmans & van der Woude, 2000). To ascertain whether the regulation observed upon constitutive expression of *stdEF* was indeed caused by HdfR we performed qRT-PCR of several genes in wild type and *hdfR*-null genetic backgrounds (**Figure C2. 8A**). Despite the different expression levels found in wild type and Δ*hdfR* backgrounds, the pattern of expression remained the same in both backgrounds, suggesting that StdEF-mediated global regulation is independent of HdfR.

It has been described in *E. coli* that HdfR binds to the *flhDC* promoter repressing its expression (Ko & Park, 2000). To investigate whether a similar mechanism might operate in our system so that *stdEF*-dependent regulation on *flhDC* might require HdfR, additional β-galactosidase assays were performed in appropriate genetic backgrounds using a *flhC*::*lac*Z transcriptional fusion. As observed in (**Figure C2. 8B**), the β-galactosidase activity of the *flhC::lacZ* fusion increased slightly in a *hdfR* null mutant as described for *E. coli*. However, constitutive expression of *stdEF* caused a similar decrease in *flhC:lacZ* activity both in the wild type and in a Δ*hdfR* background. We thus conclude that StdEF-mediated regulation of the *flh*C promoter is independent of HdfR.

Finally we also examined whether conjugation, another traitt regulated by StdEF, was HdfR-dependent. As observed in **Figure C2. 8C**, similar rates of plasmid transfer were observed in wild type and *hfd*R-null genetic backgrounds when these strains were used as donors. This observation rules out the involvement of HdfR in conjugation control.

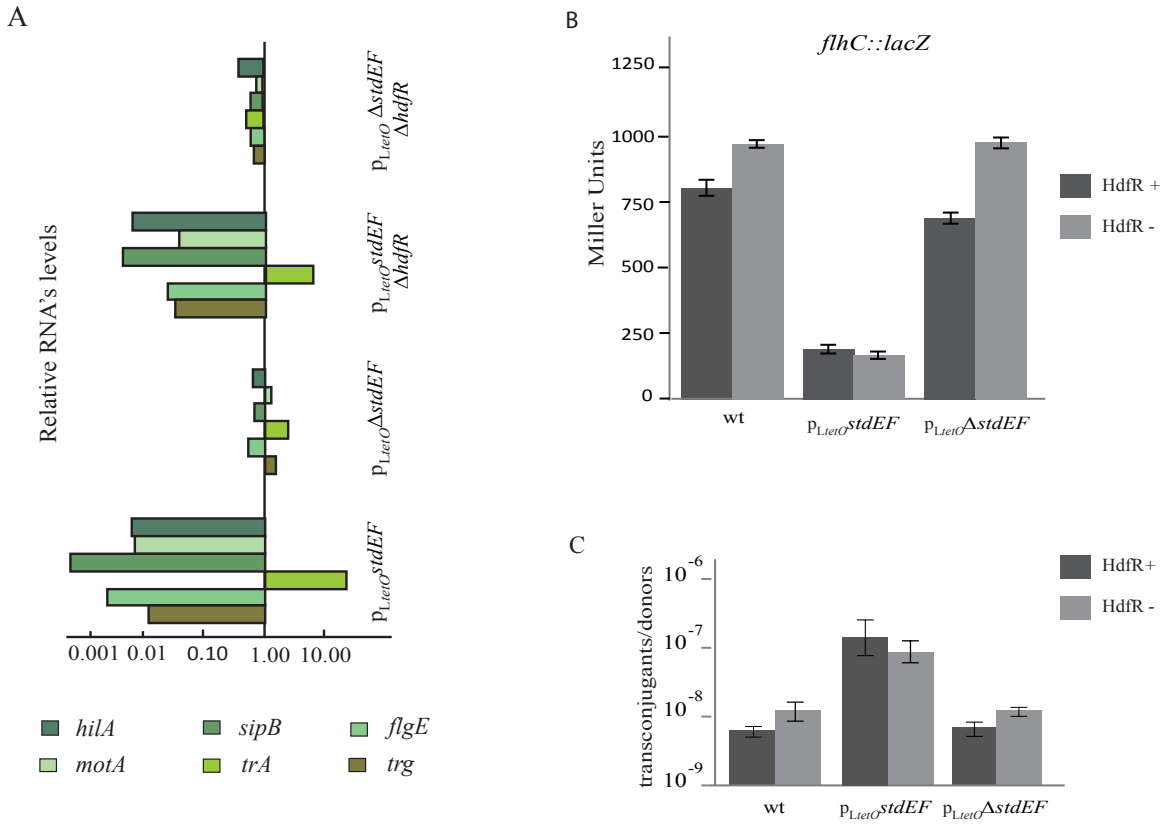

Figure C2. 8 Role of HdfR in StdEF-mediated regulation. A. RNA levels for StdEF-regulated genes in a Δ*hdfR*-null genetic background. Data are normalized to the RNA level obtained in wild type *Salmonella* cells (Value for wild type = 1; not shown). A representative experiment is shown. **B.** β-galactosidase activity of a *flhC::lacZ* fusion in wild type and Δ*hdfR*-null backgrounds. **C.** Conjugation assays using wild type and Δ*hdfR*-null donors. Data are averages and standard deviations from 3 independent matings.

StdEF regulation of SPI-1 and conjugation is independent of FlhDC.

Keeping in mind the transcriptomic data described above, another possible regulator that might control the plethora of genes regulated by StdEF was FlhDC. The *flhDC* operon is essential for transcription of all the genes in the flagellar cascade. The two operon products, FlhD and FlhC, are known to act together in an $FlhD_2FlhC_2$ heterotetramer which is the master regulator of the flagellar regulon (Claret & Hughes, 2000; Ikebe et al., 1999; Liu & Matsumura, 1994). Regarding invasion, StdEF-mediated regulation of SPI-1 expression requires post-transcriptional decrease of *hilD* mRNA. Although the actual mechanism is unknown (López-Garrido & Casadesús, 2012), it has been described that FlhDC regulates SPI-1 expression through FliZ (Chubiz et al., 2010). Because FlhDC down-regulates FliZ, it was conceivable that StdEF-mediated regulation of SPI-1 genes might be mediated by

FlhDC. β-galactosidase assays were performed in order to determine whether StdEF-mediated regulation of SPI-1, *tra*, and flagellar genes depend on FlhDC. For this purpose, a Δ*flhC* genetic background was used. Data presented in **Figure C2. 9** indicate that SPI-1 downregulation mediated by StdEF is independent of FlhDC. The same conclusion was obtained for regulation of the *tra* operon. However, as expected, flagellar regulation requires FlhDC.

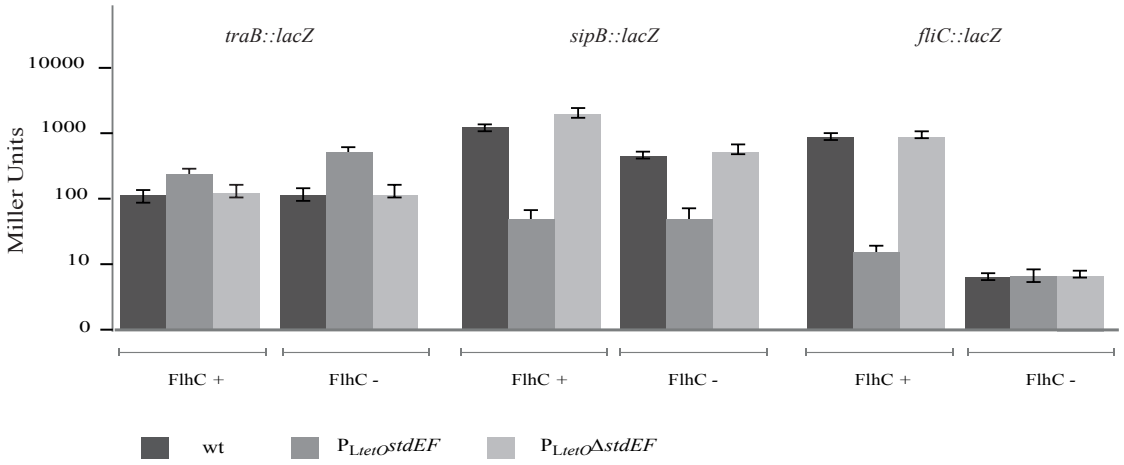

Figure C2. 9 β-Galactosidase activity of *traB::lacZ, sipB::lacZ* and *fliC::lacZ* fusions in different genetic backgrounds.

Given that, neither HdfR, nor FlhDC mediate the plethora of processes affected by *stdEF* and knowing that StdF binds the *std* promoter region (Chapter 1), we do not discard, that these molecules might have a direct involvement in the global regulation. Therefore, we performed Chromatin Immunoprecipitation followed by sequencing (ChIP-seq) analysis to probe this hypothesis.

ChIP-seq analysis of *Salmonella enterica* cells constitutively expressing StdEF-3xFLAG.

The strain used for the ChIP-seq experiment expressed *stdEF* under the control of P_{LtetO} (like the strain used in transcriptomic analysis) and contained an StdF variant labelled at its C-terminal end by a 3xFLAG epitope (P_{LtetO}*stdEF*-3xFLAG; SV7850). This construction, depicted in **Figure C2. 10**, permitted detection of StdE with a cognate anti-StdE antibody, and of StdF using an anti-FLAG antibody. Because StdE is unaltered and StdFx3FLAG remains functional, transcription of the *stdEF* regulon was unaffected.

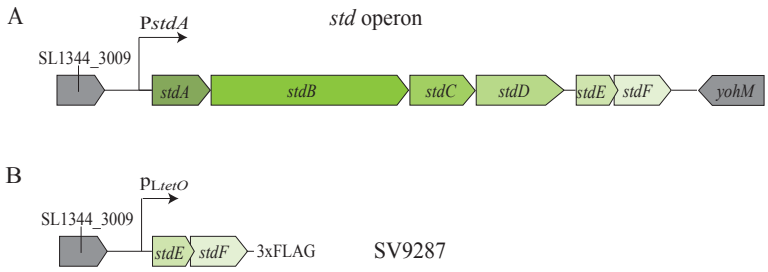

Figure C2. 10 Diagram of the strain used for ChIP-seq analysis (P_{LtetO}*stdEF*-3xFLAG).

Chromatin immunoprecipitation was performed with cultures grown to stationary phase until OD_{600}~2 (the same conditions used for microarray analysis). Bound proteins were crosslinked to DNA by formaldehyde treatment to form covalent unions. Cells were lysed and chromatin was sheared. Fragmentation of chromatin is a key point of ChIP sequencing since 300 bp fragments are required for the preparation of an optimal sequencing library, and for sequencing of the immunoprecipitated fragments. After immunoprecipitation of the specific proteins (and their bound DNA), de-crosslinking of the sample is performed to discard the proteins obtaining naked DNA fragments for the sequencing step. It is worth noting that the crosslinking step anchors the proteins to the bound DNA but also locks protein-protein interactions. Therefore our assays cannot discriminate between direct binding of the protein to DNA or indirect binding (through another protein). The controls used for the assay were: (i) total sheared and decrosslinked chromatin and (ii) mock IP sample. The mock IP is a sample that follows the same protocol as the IP sample but without adding the antibody. Despite the fact that the results from both controls were similar, we decided to use

the mock IP control for analysis of results because we considered it more reliable than the total sheared chromatin.

From the results shown in **Supplementary Table SM.2** we concluded that StdE and StdF are able to bind to multiple sites in the *Salmonella* genome. **Figure C2. 11** indicates the numbers of binding sites for StdE, StdF and for both StdE and StdF. StdE binds to 316 sites and StdF binds to 166 sites. StdE and StdF share the 20% of the sites. A higher number of DNA sequence reads were found upon inmunoprecipitation with the specific StdE antibody, suggesting that StdE binds to the *Salmonella* genome more efficiently than StdF, and that they bind in an independent manner.

Figure C2. 11 Number of StdE, StdF and both StdE and StdF binding sites along *Salmonella enterica* genome.

Looking at the binding sites for StdE and StdF, we observed that some of such sites may permit the interpretation of the data provided by the transcriptomic analysis. In other words, the StdEF binding patterns might help to explain StdEF-mediated regulation of a variety of physiological processes (e. g., by direct binding of the proteins to promoters or regulatory regions of genes). However in **Supplementary Table SM.2** we can observe that there are also binding sites that do not seem to be involved in transcriptional regulation. A tentative explanation may be that the amount of StdE and StdF proteins present in our system is relatively high, and this might produce non-specific or non/physiological bindings to the DNA. An alternative possibility is that regulation might occur under conditions different from those used in this Thesis for transcriptomic analysis.

Repression of the flagellar master operon by StdE and StdF binding

As shown in **Figure C2. 12**, a significantly higher number of DNA sequencing reads were found in the promoter region of the *flhDC* genes for both StdE and StdF immunoprecipitations. As mentioned above, *flhDC* is the master operon for flagellar regulation (Kutsukake et al., 1990), and we have observed that StdEF–mediated regulation of flagellar genes is fully dependent on FlhC (**Figure C2. 9**). These results suggest that transcription of the *flhDC* operon may be regulated by direct StdE and StdF binding to its promoter.

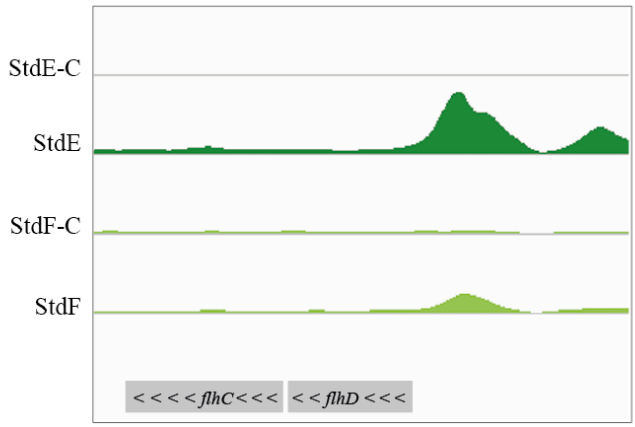

Figure C2. 12 StdE and StdF regulation of the flagellar system through FlhDC. Chip-seq data show StdE and StdF binding peaks at the *flhDC* promoter. StdE-C and StdF-C denote control reactions (mock IP) for StdE and StdF inmunoprecipitations, respectively,

Regulation of chemotaxis by StdE and StdF is mediated by FlhDC

Transcriptomic data described above showed that StdE and StdF repress the *che* operon, as well as the *trg* and *aer* genes involved in chemotaxis and aerotaxis. Analysis of the ChIP-seq results did not show a binding peak close to those operons. However, it is well known that chemotaxis regulation is dependent on the *flhDC* master regulator (Chilcott & Hughes, 2000). Based on this fact, we wondered whether regulation by *StdEF* might be mediated by FlhDC. To investigate this possibility, quantitative RT-PCR was performed to evaluate the mRNA levels of genes differentially expressed in the presence of StdE and StdF according to the transcriptomic data (*cheA*, *cheM*, *che*, and *trg*) in $FlhC^{+}$ and $FlhC^{-}$ backgrounds.

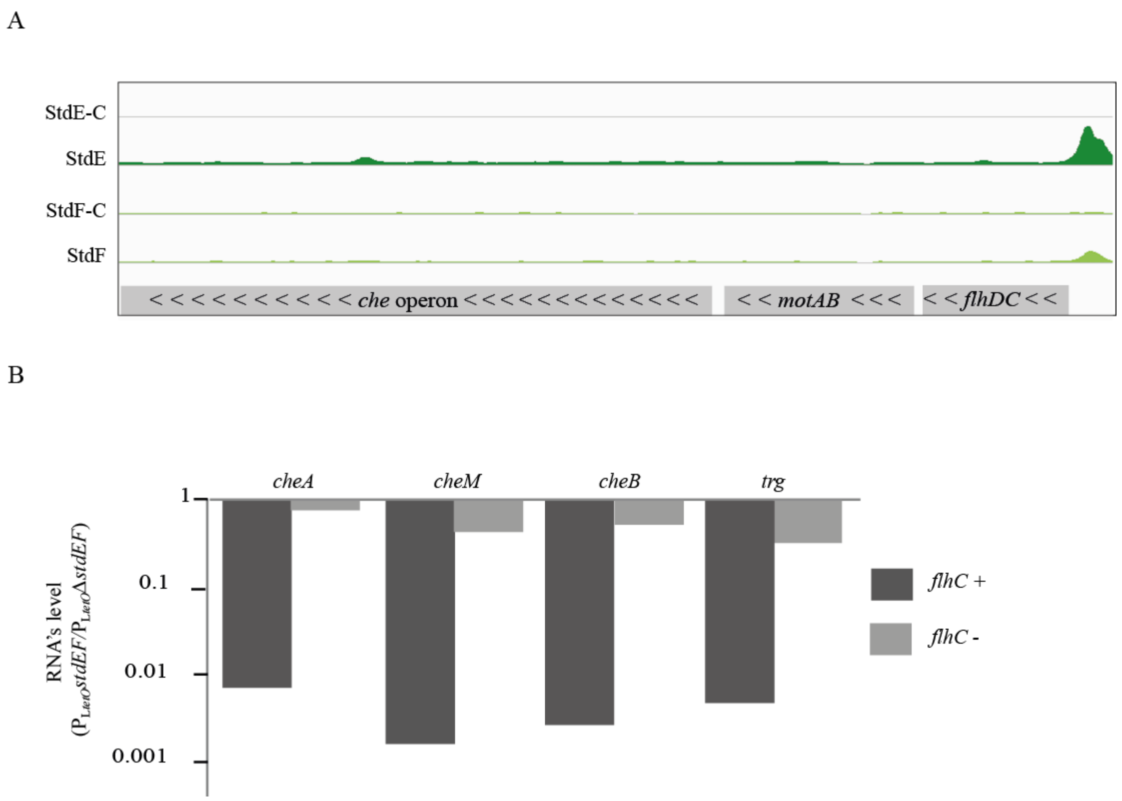

Figure C2. 13 Regulation of chemotaxis by StdE and StdF is mediated by *flhDC*. A. ChIP-seq data shows no relevant peaks surrounding the *che* operon. **B.** Ratios of the mRNA levels of genes involved in chemotaxis between the strains P_{LtetO}*stdEF* and $P_{LtetO}\Delta$*stdEF*. RNA levels were determined for at least 3 biological replicates and a representative experiment is shown.

Data presented in **Figure C2. 13B** confirm that regulation of chemotaxis by StdEF is actually mediated by the flagellar master regulator FlhDC. This observation reminds of results described in both *Salmonella* and *E. coli*, where FlhDC is the transcriptional activator of σ^{28} *(fliA)* (among other second class operons). FliA drives transcription of class 3 flagellar operons, such as *tar-tap-cheRBYZ* and *motAB-cheAW* (Chilcott & Hughes, 2000). Similar observations have been made in other motility or chemotaxis related genes such as *trg* and *aer* (Ghigo, 2001; May & Okabe, 2008) whose transcription is dependent on RpoF (*fliA*-σ^{28}) (Fitzgerald et al., 2015).

StdE binds the *hilD*-coding region

StdEF downregulate SPI-1 expression, and downregulation takes place through *hilD* at the post-transcriptional level (López-Garrido & Casadesús, 2012). We examined the data obtained from the ChiP-seq analysis in the SPI-1 region, and we found that only StdE appears

to bind within the region, and that binding occurs at the end of the *hilD* coding region (**Figure C2. 14**). Based on the data from the ChIP-seq analysis (**Supplementary Table SM.2**) other minor binding peaks in SPI-1 region were found in *sipB* and *sipC* genes, which encode effector proteins. Outside SPI-1, no binding was detected in genes known to code for SPI-1 regulators (e. g., *hilE*).

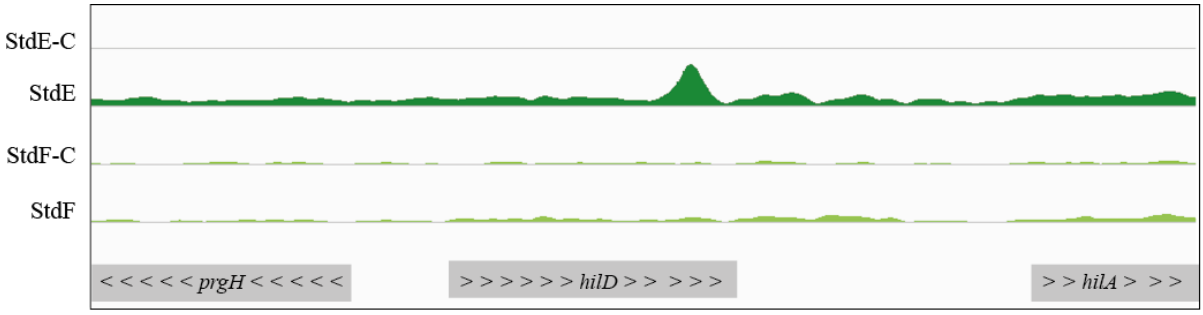

Figure C2. 14 StdE binding to the *hilD* coding region.

Previous work on StdEF-mediated regulation of SPI-1 (López-Garrido & Casadesús, 2012) concluded that regulation involved post-transcriptional repression of HilD, and that the main player in this regulation was StdE. These data correlate well with our ChIP-seq data: only StdE seems to bind the region. Hence, we considered the possibility that binding of StdE to the hilD coding region might somehow affect the stability of hilD mRNA. In these experiments, expression of hilD was driven by the P_{LtetO} promoter. This choice was based upon the fact that the levels of hilD mRNA upon stdEF constitutive expression were very low, thereby making difficult the calculation of hilD mRNA half-life. We first verified that the strain P_{LtetO}*stdEF* P_{LtetO}*hilD* was able to maintain the regulation pattern of SPI-1 genes in the same way as when hilD was transcribed from its own promoter. Data in Figure C2. 15A show that regulation occurs as expected, and that StdEF-mediated regulation of SPI-1 genes does not require hilD to be transcribed from its native promoter. Although the *hilD* mRNA level was lower in the strain that constitutively expressed StdEF, the stability of hilD mRNA was similar in both strains (t ½ for P_{LtetO}*stdEF* P_{LtetO}*hilD* and P_{LtetO}Δ*stdEF* P_{LtetO}*hilD* is 2,98 and 2,82 min respectively), ruling out a mechanism of regulation involving hilD mRNA decay (Figure C2. 15B).

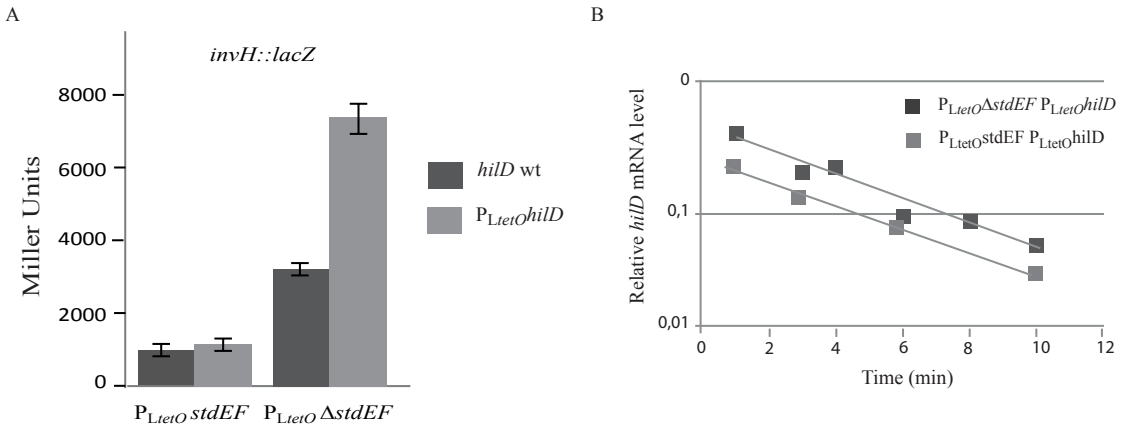

Figure C2. 15 Stability of *hilD* mRNA. A. β-Galactosidase activity of the *invH::lacZ* fusion. **B.** Stability of *hilD* mRNA in P_{LtetO}Δ*stdEF* P_{LtetO}*hilD* and P_{LtetO}*stdEF* P_{LtetO}*hilD* strains. Values are averages from 3 independent qRT-PCR reactions. Error bars are not shown because the estándar deviations were extremely small.

The mechanism of post-transcriptional regulation of *hilD* upon StdE binding to its coding sequence cannot be easily explained. One speculation is that StdE binding at the downstream portion of the coding region might interfere with the DNA-RNA hybrid formed during transcription, reducing the transcription rate. Another possibility is that binding of StdE to the hilD coding region might regulate the expression of a putative antisense RNA, resulting in a decrease of hilD mRNA

Control of pSLT conjugal transfer by StdEF

Transcriptomic data (**Supplementary Table SM.1)** and phenotypic assays (**Figure C2. 4C)**, show increased conjugal transfer of the *Salmonella* virulence plasmid (pSLT) if the donor strain constitutively expresses *stdEF* (SV8141). This increase in plasmid transfer may be correlated, at least in part, with up-regulation of *tra* operon genes upon StdEF constitutive synthesis.

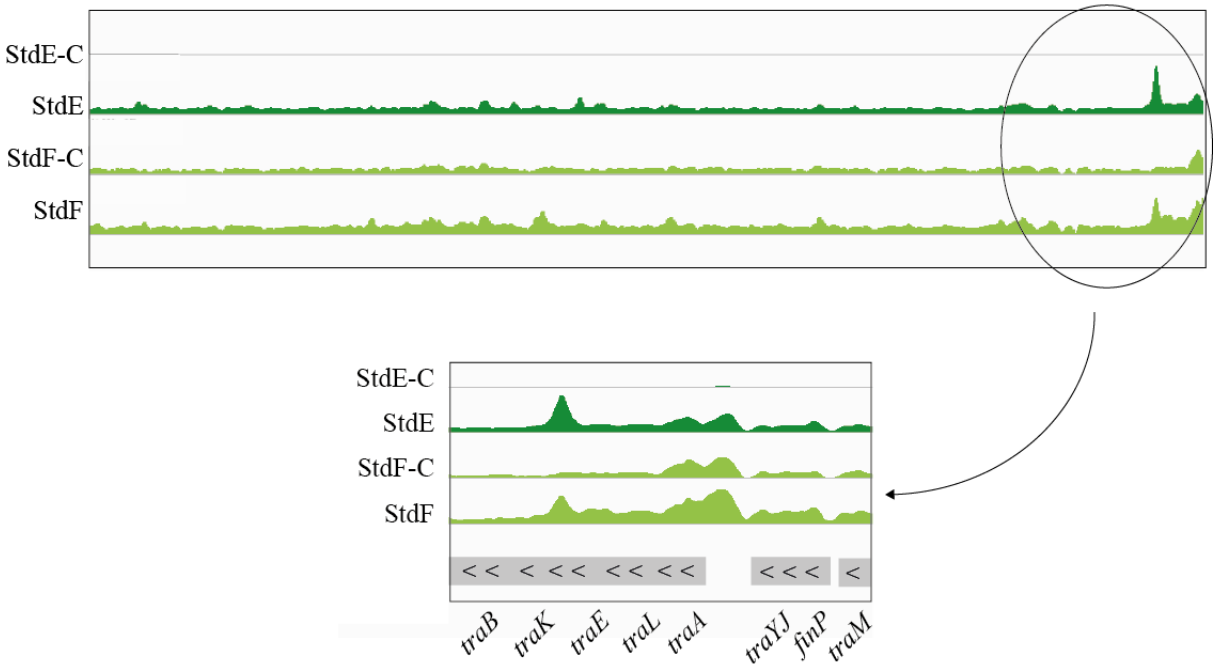

Figure C2. 16 ChIP-seq data showing pSLT plasmid region. In the upper part, peaks along the *tra* operon are shown. StdE and StdF binding peaks are detected along *tra* operon. In the lower part of the figure, the *tra* operon region is zoomed-in.

A closer look to the ChIP-seq data in the *tra* operon shows an increase of StdE and StdF binding peaks, mainly upstream of *traA* and upstream of *traK* (**Figure C2. 16**). In the microarray (**Figure C2. 17**), the most up-regulated gene in the *tra* operon is *traA* with a 24-fold increase.

Gene	Fold change P_{LtetO}*stdEF*/P_{LtetO}Δ*stdEF*
traA	24.3
traY	16.0
traL	11.3
traE	10.6
traP	6.5
traB	3.0

Figure C2. 17 Expression of *tra* genes in P_{LtetO}*stdEF* vs P_{LtetO}Δ*stdEF* strains.

Based on the strength of regulation, the binding peak closer to *traA* gene might be functional, and binding to the *traA* promoter might activate transcription. Moreover, *traB* appears as the less up-regulated gene in the *tra* operon, which suggests that the peak upstream *traK* and *traB* is not functional. StdEF-mediated regulation might thus involved the *traA* promoter.

StdE and StdF binding sites identified in *hdfR* and *std* promoters

Transcriptomic data and functional validation congruently indicate that increased StdEF synthesis in the strains that carry the P_{LtetO}*stdEF* construct increase the level of *hdfR* expression, the transcriptional regulator of the *std* operon. Therefore, *std* expression increases the amount of the transcription factor needed for keeping the transcription of *std* operon, generating a self-regulatory positive loop (see Chapter 1).

ChIP-seq upon StdE immunoprecipiation identified a peak upstream of the *hdf*R gene (**Figure C2. 18**), suggesting a direct activation of transcription by the StdE binding to the *hdfR* promoter.

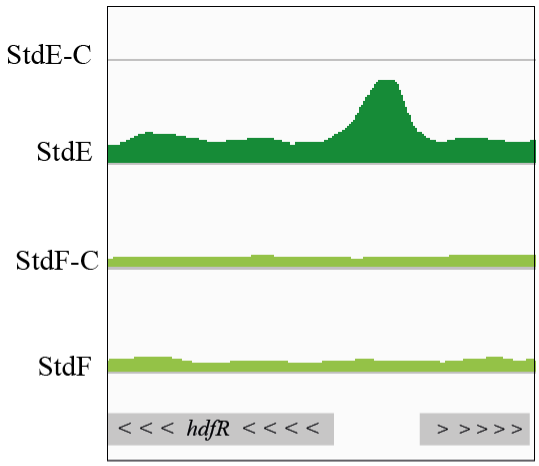

Figure C2. 18 ChIP- seq data obtained in the *hdfR* locus.

Regarding *std* autoregulation, a binding peak was found upstream of the *std* promoter (**Figure C2. 19**), as expected according to the results presented in Chapter 1. However, we were lucky to identify this binding site, since the P_{LtetO}*stdEF*-3xFLAG strain used for the ChIP-seq assay was constructed placing the P_{LtetO} promoter upstream of the *stdE* gene and deleting the remaining sequences of operon upstream of *stdE* (which included the *std* promoter). The binding site identified by ChIP-seq is located 40 nt upstream of the deletion

fragment, and 80 nt upstream of the first GATC site, suggesting a mechanism of autoregulation in which binding of StdF in this region favours *std* transcription. This result confirms previous data from the genetic screen (see Chapter 1) that outlined the importance of StdF for autoregulation of the *std* operon. It is interesting to point out that the binding site upstream of the *std* operon is the only one case in which StdF binds independently of StdE. The fact that StdF binding sites are mostly shared by StdE suggests a mechanism in which StdF binding to DNA might be accessory for regulation. An alternative possibility is that StdF does not bind directly DNA and its presence at the site is due to an interaction with other DNA-binding proteins (i.e., HdfR). For instance, StdF might act as a chaperone to facilitate binding of the regulator to cognate DNA.

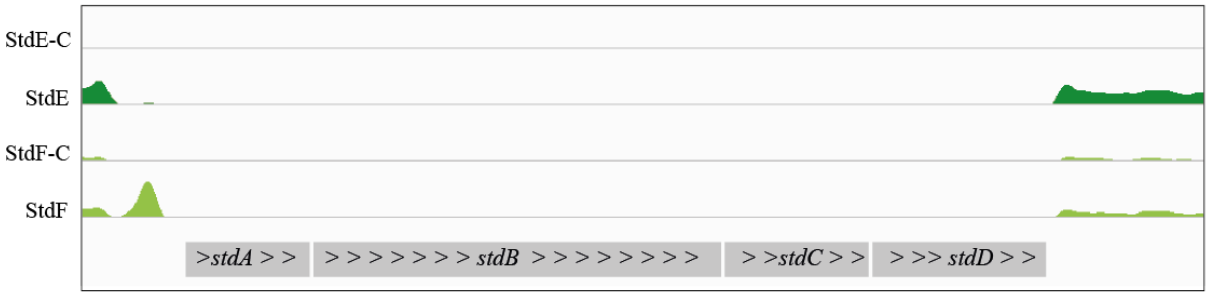

Figure C2. 19 ChIP-seq data showing StdF binding to *std* promoter region.

Based on the ChiP-seq data, together with the results presented in Chapter 1, we propose a mechanism in which StdE binding to the *hdf*R promoter activates its expression, therefore maintaining HdfR levels high enough to allow expression from the *std* promoter, and to keep the autoregulatory loop active. StdF binding to the *std* promoter favours its transcription, and the accessory role of StdF might be either assisting HdfR binding, favouring a positive regulation, or by obstructing Dam methylation, which represses expression, therefore resulting in the positive regulation observed. Based in the results presented in **Figure C2. 20** it might be suggested that StdF is involved in HdfR binding. In a wild type strain, both HdfR and StdF are essential for expression of the *std* operon. In a Dam$^-$ mutant, were there is no methylation, HdfR is essential but also StdF. Hence, when no blocking action is needed, StdF seems to be still essential for *std* expression.

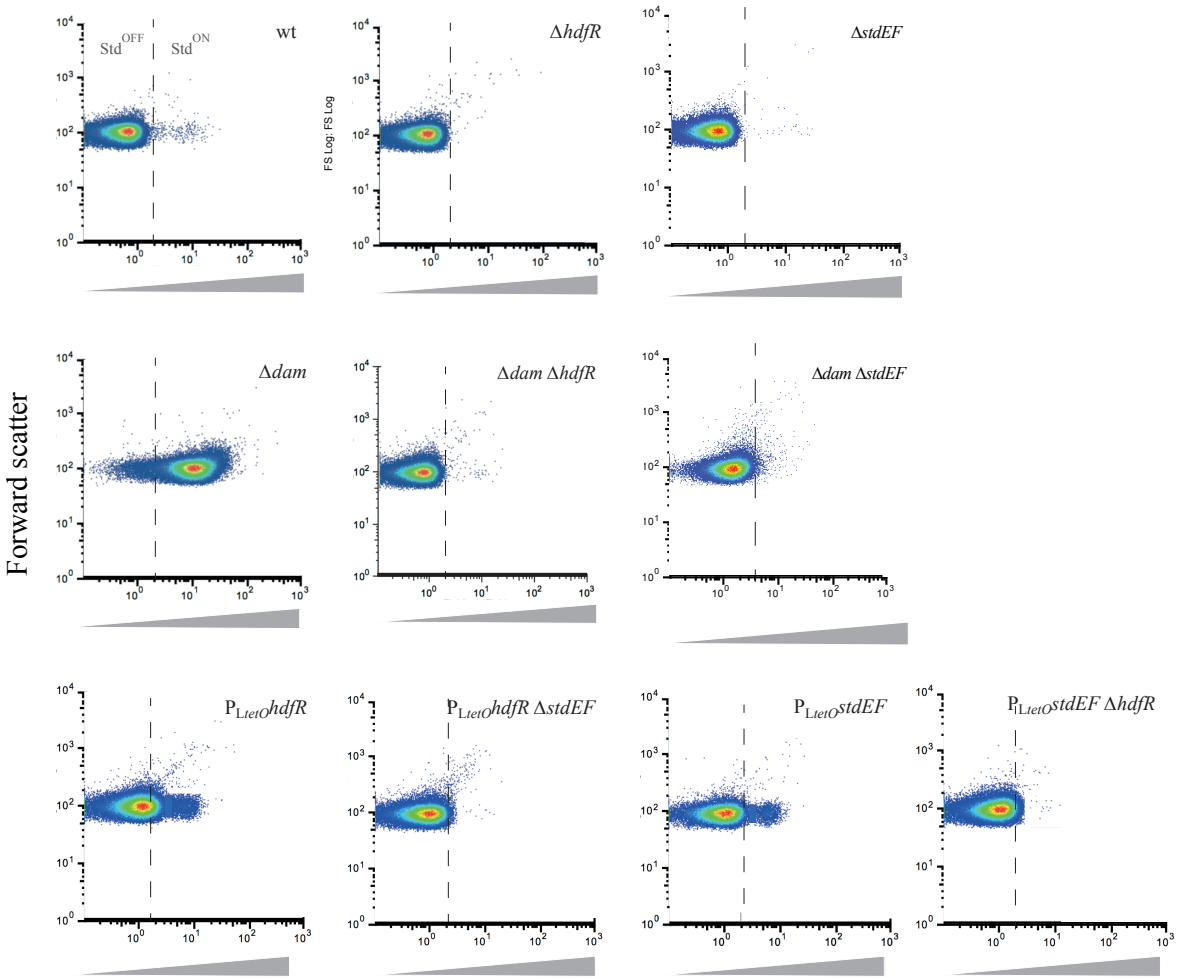

Figure C2. 20 Role of StdF in *std* activation. Heat maps represent GFP fluorescence intensity in *S. enterica* strains carrying an *stdA::gfp* fusion in different backgrounds.

Based on these results and others described in Chapter 1, we confirm that StdE and StdF have a crucial role in autoregulation the *std* operon (**Figure C2. 21**). The involvement of these regulators takes place at two different levels. StdE binds the *hdfR* promoter region and increases *hdfR* transcription. It would be interesting to figure out if the increase is due to an active StdE action over the *hdfR* promoter or to blocking HdfR negative loop. On the other hand, StdF binds to the *std* promoter and may act as an auxiliary protein that cooperates with HdfR to activate *std* transcription.

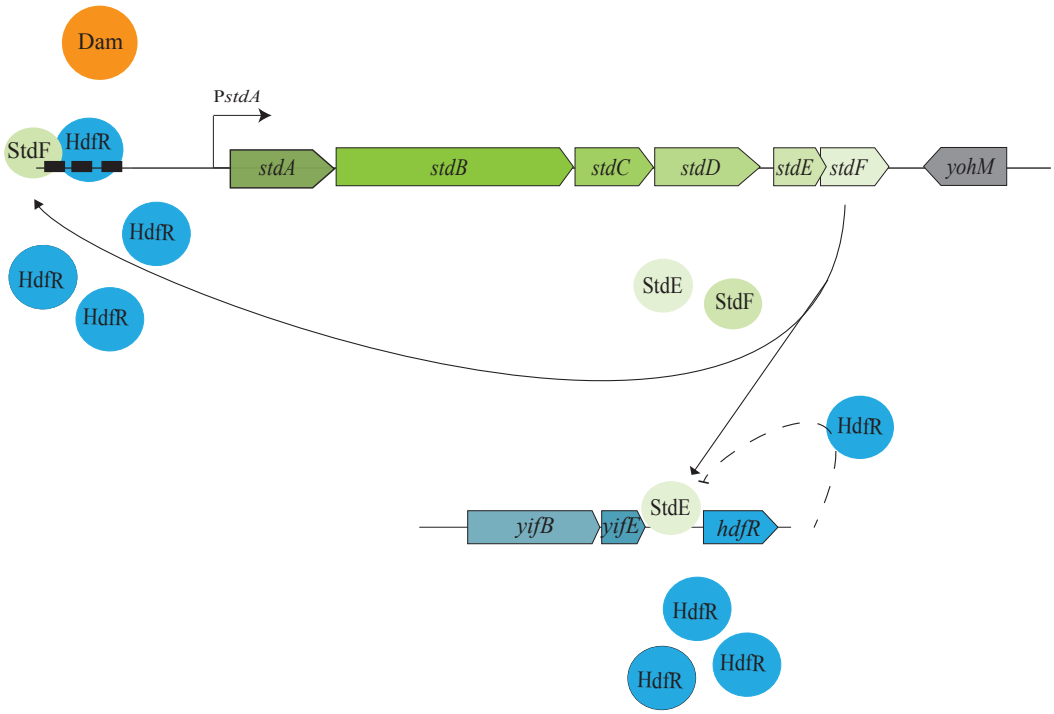

Figure C2. 21 Tentative model of *std* autoregulation. Positive effects are indicated by lines ending in arrowheads, whereas blunt lines indicate negative regulatory effects. Dashed lines indicate regulations that do not apply under the diagram circumstances. The three black squares represent the three GATC sites in the regulatory region of the *std* operon, that in the circumstances detailed above would be non-mehylated.

DISCUSSION

Variable and unpredictable environments can confer selective value to adaptive phenotypes. Traditionally it was assumed that bacteria adapt to changing environments by transcriptional and postranscriptional regulation. According to this view, regulatory networks integrate signals of environmental alterations, and an improved phenotype may be generated. These adaptive procedures are slow, and in certain harsh environments can be insufficient (Turner et al., 2009). During the last decade, single cell analysis has provided examples indicating that, in certain clonal populations of bacteria, not all the cells show the same phenotype under the same selective pressure. Bacterial phenotypic heterogeneity can originate from genetic changes, including programmed genetic rearrangement (Silverman et al., 1979) and contraction or expansion of DNA repeats at genome regions known as contingency loci (Moxon et al., 1994; 2006). Phenotypic heterogeneity can also be epigenetic, without any change in the DNA sequence (Casadesús & Low, 2013). Phenotypic variability can increase the fitness of the bacterial population either by permitting division of labor or by bet-hedging (anticipation of future challenges) (Veening et al., 2008). This view is not merely intuitive: game theory analysis supports it (Beaumont et al., 2009; Grafen, 1999; Wolf et al., 2005).

Relevant examples of non-genetic heterogeneity in isogenic populations involve envelope structures such as fimbriae, flagella, and the LPS O-antigen (Cota et al., 2015; Cummings et al., 2006; Kingsley et al., 2002; Nicholson & Low, 2000). In the Dam-regulated *std* fimbrial operon of *Salmonella enterica* (Balbontín et al., 2006), the existence of subpopulations of Std^{ON} and Std^{OFF} cells was observed in Dam^- and $SeqA^-$ mutants (Jakomin et al., 2008). Results obtained in this Thesis indicate that this phenotypic heterogeneity occurs also in the wild type strain, where a large subpopulation Std^{OFF} cells and a small subpopulation of Std^{ON} cells are found. The existence of bistability is also supported by the visualization of the StdA fimbrial protein by immunofluorescence microscopy in a small fraction of the population. Bimodal expression had been previously reported for other fimbrial operons such as *lpf* and *pef* (Kingsley et al., 2002; Nicholson & Low, 2000; Norris et al., 1998).

The transcriptional factor HdfR had been shown to be essential for *std* expression in a Dam^- background (Jakomin et al., 2008), and data presented in this Thesis indicate that HdfR activates *std* transcription in the wild type strain as well. In fact, constitutive expression of *hdf* increases the size of the Std^{ON} subpopulation. Given the importance of HdfR availability for *std* expression, one might think that lack of s*td* expression in the wild type (which occurs in the majority of cells) might be due to HdfR shortage. Far for being the bottleneck, the

amount of HdfR in the wild type strain is roughly the same as in a Dam⁻ mutant where *std* expression is considerably higher. The maintenance of a steady level of HdfR seems to be due, at least in part, to the existence of an auto-regulatory loop that maintains *hdfR* expression at moderate levels. Like other LysR-type transcription factors, HdfR undergoes autogenous transcriptional repression. This loop operates both in the wild type and in a Dam⁻ mutant. In the latter, however, increased expression of *stdEF* increases the amount of HdfR because *hdfR* transcription is up-regulated by StdEF (see below).

Besides Dam and HdfR, SeqA (Jakomin et al., 2008) and RosE (Chessa et al., 2008) have been described as regulators of the *std* transcription. While searching for additional regulators of *std* expression, circumstantial evidence suggested that Std products might be activators of *std* transcription, providing a positive feedback loop for *std* operon control. Constitutive expression of the *stdEF* genes increases the expression of the *std* operon and the size of the Std^{ON} subpopulation. On the contrary, absence of the *stdEF* genes decreases the size of the Std^{ON} subpopulation. Based on these observations, we conclude that the products of the two downstream genes of the *std* operon (StdE and StdF) are positive regulators of *std* transcription. However only StdF seems to bind the *std* promoter (see below).

With these observations in mind, a model for *std* operon control may be proposed. The Dam methylase, the transcription factor HdfR, and the StdF protein may compete for binding to the *std* regulatory region. In fact, HdfR binds the *std* UAS when the GATC sites are non-methylated, protecting them from methylation. As mentioned above, increased levels of HdfR increase both the size of the Std^{ON} subpopulation and the mean fluorescence of the population. In our tentative model, the *std-hdfR-std* feedback loop can be expected to generate amounts of HdfR high enough to unbalance the pulse between Dam and HdfR for occupying the *std* regulatory region. The reason why HdfR excludes the Dam methylase only in a fraction of cells remains unknown but a model involving the StdF gene product can be presented. The StdF protein binds the *std* promoter region, and two observations suggest that it may cooperate with HdfR, rather than hindering Dam methylation: (1) the presence of the StdEF products is still necessary to hinder Dam methylase activity when the expression levels of *hdfR* are high enough (*std* expression is higher in a P_{LtetO}*hdfR* background than in a Δ*dam* background); and (2) in the absence of Dam methylation the *stdEF* genes are still necessary for *std* transcription. Furthermore, analysis of *std* expression in different backgrounds (wild type, Dam⁻, and P_{LtetO}*hdfR*) reveals that the Std^{ON} subpopulation is always detected when

StdE and StdF are present, suggesting that StdF binding to the *std* UAS may be the key factor to generate Std^{ON} cells.

To better understand the mechanisms that converge at the regulatory region, determination of the methylation state of the *std* promoter in Std^{OFF} and Std^{ON} cells might provide relevant information. However, a handicap emerges here because of the existence of two phenotypic subpopulations, one of them tiny. In the major subpopulation (Std^{OFF}), the three GATC sites in the regulatory region of the *std* operon are fully methylated. Based on the changes in *std* expression observed when the main regulators are overexpressed or absent, a different pattern can be expected for the Std^{ON} subpopulation. This possibility is reinforced by the decrease of the Std^{ON} subpopulation upon DNA methylase overproduction. However, the methylation state of the *std* UAS in the Std^{ON} subpopulation remains to be determined.

If synthesis of StdE and StdF is low and noisy, differences from cell to cell may explain why HdfR-mediated activation and concomitant formation of nonmethylated GATCs occurs in certain cells only. An observation that may support this view is the detection of a weak internal promoter upon measuring the fluorescence activity of an *stdE::gfp* fusion in the presence and in the absence of the main promoter. Several attempts have been made while preparing this Thesis to identify the putative internal promoter. However, cloning of internal *std* fragments onto the promoter-probe vector pIC552 did not reveal the existence of an active promoter. Inconclusive results were also obtained by 5´RACE experiments performed in both the wild type and a Dam mutant. The experiments performed using the promoter-probe plasmid pIC552 may have failed due to the existence of unknown repressors that may control *stdE* expression. In the case of 5'RACE, expression of the *stdE* gene in the wild type strain may be too low to detect the RNA molecules. Unfortunately, a Dam^{-} mutant cannot be used in 5'RACE experiments because in this background the main *std* promoter is active.

Autoregulation of the *std* operon by the StdE and StdF products is not a unique example: the involvement of fimbrial products in the regulation of fimbrial synthesis had been previously reported. To give just an example, expression of the major fimbrial subunit of type 1 fimbriae of *Salmonella enterica* (*fimA*) is positively regulated by FimY and FimZ (Saini et al., 2009; Tinker & Clegg, 2000), and FinM binds the *fimA* promoter (Yeh et al., 1995).

Aside from the repressors of the *std* operon already known (Dam methylation, SeqA and RosE), we have identified a new repressor, RcsB, the response regulator of the RcsCBD

signal transduction system. RcsB represses *std* expression. This regulation takes places not only in a Dam⁻ background but also in the wild type strain, decreasing the fraction of Std^{ON} cells in the population. Based on the results presented here, this regulation is not dependent on Dam methylation, since repression is observed both in the presence and in the absence of Dam methylation. RcsB may exert transcriptional regulation by binding directly to the *std* promoter region, and *in silico* analysis has identified a putative binding site for RcsB near the -35 module of the *std* promoter. A footprint assay will be necessary to confirm the existence of RcsB binding to the putative binding site identified. The significance of RcsBCD-mediated repression of the *std* operon is difficult to grasp, even in a speculative manner, as the environmental signals that activate the RcsBCD signal transduction system remain largely unknown (Majdalani & Gottesman, 2005).

Fractionation assays have identified StdE and StdF as cytoplasmic proteins, and *in silico* information reveals that StdE and StdF are homologues of known transcriptional regulators. StdE shares 40-50% identity with the transcriptional activators GrlA from *E. coli* and CaiF from *Enterobacter cloacae*. In turn, StdF is related to the SPI-1 *Salmonella* protein SprB, a transcriptional regulator that represses the *hilD* promoter and activates the *siiA* promoter (López-Garrido & Casadesús, 2012). Homology with transcriptional regulators correlates well with our results, although no DNA binding motifs were identified *in silico.*

In 2012, López-Garrido & Casadesús proposed that the *std* operon may be the link between Dam methylation and SPI-1 regulation: the downregulation of SPI-1 expression observed in Dam⁻ mutants (Balbontín et al., 2006) is suppressed upon deletion of two *std* genes, *stdE* and *stdF* (López-Garrido & Casadesús, 2012). This antecedent, combined with the fact that this Thesis has uncovered an active role of StdE and StdF as transcriptional activators of their own operon, led us to wonder whether these products of the *std* operon might have other roles in *Salmonella enterica*. To our surprise, microarray analysis revealed that StdE and StdF are global regulators of transcription, mainly as repressors. Constitutive expression of *stdE* and *stdF* downregulates not only SPI-1 expression as previously reported (López-Garrido & Casadesús, 2012) but also represses loci involved in motility and chemotaxis. On the other hand, StdEF activate transcription of the pSLT *tra* operon and, interestingly, of *hdfR*.

Since StdEF activate *hdfR* expression and LysR-type transcription factors are often global regulators, we could not role out the possibility that some or all the global regulatory activities of StdE and StdF were actually performed by HdfR. However, we obtained

evidence that HdfR is not a global regulator but merely one of the many targets within the StdEF regulon. With *hdfR* transcriptional activation, the *std* operon may ensure its own transcription as HdfR is needed for *std* transcription. This regulatory pattern generates a positive feedback loop for *std* expression. A tempting speculation is that StdEF may increase the level of HdfR through direct binding of StdE to the regulatory region of the *hdfR* gene, thereby impairing autogenous repression by HdfR.

Another possible intermediary in StdEF-mediated global regulation is the FlhDC master regulator, known to control invasion, motility and fimbriation (Saini et al., 2010). Furthermore FliZ, a product of FlhDC regulatory cascade, has been described as a postranscriptional regulator of SPI-1 through *hilD* (Chubiz et al., 2010). However, our results discard FlhDC as the connector between StdEF and their targets. FlhDC does regulate the flagellar system as well as chemotaxis (as expected), but none of the other processes under StdEF control.

Because the potential intermediary factors considered above were not involved in global regulation by StdEF and we had previously observed that StdF acts as a transcription factor promoting *std* expression, we considered the option of direct regulation by StdEF. ChIP-seq analysis was thus performed to ascertain whether StdE and StdF are DNA-binding proteins. This analysis confirmed that StdE and StdF have DNA-binding ability, thus supporting the possibility that they may directly regulate gene expression. StdE binds twice more frequently than StdF but both proteins share 20% of the binding sites. This kind of assay does not distinguish direct binding from indirect binding. Hence, in certain cases we cannot rule out the involvement of both proteins in regulation.

Although we cannot propose a mechanism regarding SPI-1 regulation, we have confirmed the occurrence of postranscriptional regulation of *hilD* as previously described (López-Garrido & Casadesús, 2012). Moreover, the relevant role that we assigned to StdE in SPI-1 regulation correlates well with the data presented by Lopez-Garrido *et al*. (2012) showing that the decrease of SP1-1 regulation is stronger when StdE is present than when only StdF is present. As discussed above, the ChIP-seq assay does not allow to distinguish between direct binding of a protein to the DNA and indirect binding through another protein. However, it would be interesting to study in depth how is it possible that StdE controls *hilD* expression by interacting with the downstream portion of its coding region. An attractive idea involves the hybrid DNA-RNA formed during transcription. StdE might impair RNA processing, thereby

affecting translation of the HilD protein. As a consequence, disturbance of SPI-1 regulation might occur, and also disruption of the *hilD* self-regulatory loop. Regulation of the expression of a putative antisense RNA can be also considered, but *in silico* analysis based on data provided by Jay Hinton's lab seem to discard the possibility of an additional transcription start site aside from those already known in the region.

Concerning the regulation of motility and chemotaxis, the ChIP-seq data raise the possibility that both StdE and StdF bind directly to the *flhDC* promoter. Regulation at the highest level in the hierarchy of the flagellar network (FlhDC) seems to explain why all the operons involved in regulation of motility appear to be under StdEF control. Regulation of chemotaxis is also controlled through FlhDC, probably involving the Class III transcription factor, FliA. *A priori*, it seems to make sense that the motility system is not active if the ability to sense the environment is not working and *vice versa*. Interestingly, we have observed that the regulation of the flagellar system mediated by Dam methylation is, at least partially, due to StdEF, in a way reminiscent of the crosstalk previously described for SPI-1 regulation.

Even though StdE and StdF appear to be mainly repressors of transcription, it is noteworthy that the conjugal transfer operon *tra* and the *hdfR* gene are activated by StdEF. We cannot propose a mechanism for StdEF-mediated regulation of conjugation, but it is clear that StdE and StdF binds massively along the *tra* operon. We cannot ascertain if one or both proteins are involved in *tra* operon control nor define the specific target(s). However, based on the expression level of the target genes, the *traA* regulatory region might be the most appropriate for regulation by StdE, StdF, or both.

At this point, and considering that the most widespread role of StdE and StdF appears to be to act as repressors of transcription, one may consider the possibility that up-regulation mediated by *stdEF* might be actually performed by repressing a repressor. We have already mentioned that StdEF-dependent regulation of *hdfR* may involve impairment of HdfR binding to its own promoter. Something similar may happen with StdEF-dependent regulation of *tra* operon. Unfortunately, among the transcriptomic data we have not found an appropriate candidate. However, we have observed that Dam methylation-dependent regulation of the *tra* operon is, as described previously for the flagellar regulation, also due to StdEF. So StdEF are involved in Dam dependent regulation of SPI-1 (López-Garrido & Casadesús, 2012), flagellar synthesis and conjugation. In the case of conjugation, StdEF-

mediated activation of *tra* might boost Lrp-mediated activation of *traJ* (Camacho & Casadesús, 2002) and counter or balance FinP-mediated repression (Camacho & Casadesús, 2005). The latter two mechanisms of conjugation control are also Dam-dependent, albeit in opposite ways: Dam methylation activates transcription of *finP* and represses transcription of *traJ*. The existence of overlapping controls acting in opposite manners may illustrate, like many other examples, the complexity of bacterial regulatory networks.

A major outcome of this Thesis work is that the study provides a novel example of phenotypic variation within a clonal population. Two bacterial subpopulations, made of fimbriated and non-fimbriated *Salmonella* cells, appear to exist. Formation of two subpopulations may be viewed as a preadaptation of *Salmonella enterica* to perform two types of infection, acute and chronic. Because Std fimbriae permit adhesion to the epithelium of the caecum (Weening et al., 2005), Std^{ON} cells will be able to colonize the large intestine, and this colonization may result in persistent or chronic infection. In turn, Std^{OFF} cells will be able to undergo invasion of the small intestine epithelium, causing salmonellosis or systemic infection. The fact that the Std^{ON} and Std^{OFF} subpopulations differ in additional phenotypic traits may contribute to adaptation by fine-tuning the interaction of each subpopulation with a specific host environment.

CONCLUSIONS

1. Populations of *Salmonella enterica* cells undergo bistable expression of the *std* fimbrial operon. The Std^{ON} subpopulation is small in the wild type (0.3-3% cells) under laboratory conditions.

2. Formation of the Std^{ON} subpopulation requires the StdE and StdF proteins, which are products of the promoter-distal genes of the *std* operon. StdE is necessary to activate transcription of the *hdfR* gene, which encodes the main transcriptional activator of the *std* operon. In turn, StdF may be necessary to facilitate binding of HdfR to the *std* promoter.

3. StdE and StdF are global regulators in *Salmonella enterica*, and regulate SPI-1 expression, flagellar synthesis, chemotaxis, biofilm formation, and conjugal transfer of the virulence plasmid.

4. StdE and StdF are DNA-binding proteins that show homology with other transcriptional regulators, and may control transcription by direct binding to DNA. An exception is the *hilD* gene, which undergoes postranscriptional regulation under StdE control. The mechanism underlying *hilD* regulation is unknown.

5. The Std^{OFF} subpopulation is able to invade epithelial cells, while the Std^{ON} subpopulation is deficient in epithelial cell invasion. This difference may endowe the Std^{OFF} subpopulation with the ability to cause salmonellosis or systemic infection. In turn, production of Std fimbriae by the Std^{ON} subpopulation may permit adhesion to the caecum, causing chronic infection.

6. As a consequence of global regulation of gene expression by StdEF, the Std^{ON} subpopulation is nonmotile, and undergoes altered biofilm formation, repression of chemotaxis genes, and activation of the conjugation system of the virulence plasmid.

7. The existence of multiple phenotypic differences between the Std^{OFF} and the Std^{ON} subpopulations may be viewed as a case of bacterial differentiation during *Salmonella* infection, perhaps as a bet hedging strategy or a division of labour.

REFERENCES

Ackermann, M. (2013). Microbial individuality in the natural environment *ISME J* 7, 465-467.

Ahmer, B. M., Tran, M. & Heffron, F. (1999). The virulence plasmid of Salmonella typhimurium is self-transmissible *J. Bacteriol.* 181, 1364-1368.

Akbar, S., Schechter, L. M., Lostroh, C. P. & Lee, C. A. (2003). AraC/XylS family members, HilD and HilC, directly activate virulence gene expression independently of HilA in Salmonella typhimurium *Mol. Microbiol.* 47, 715-728.

Andrews, S. (2010, April 25). FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Anjum, M. F., Marooney, C., Fookes, M., Baker, S., Dougan, G., Ivens, A. & Woodward, M. J. (2005). Identification of core and variable components of the Salmonella enterica subspecies I genome by microarray *Infect. Immun.* 73, 7894-7905.

Badie, G., Heithoff, D. M., Sinsheimer, R. L. & Mahan, M. J. (2007). Altered levels of Salmonella DNA adenine methylase are associated with defects in gene expression, motility, flagellar synthesis, and bile resistance in the pathogenic strain 14028 but not in the laboratory strain LT2 *J. Bacteriol.* 189, 1556-1564.

Bajaj, V., Hwang, C. & Lee, C. A. (1995). hilA is a novel ompR/toxR family member that activates the expression of Salmonella typhimurium invasion genes *Mol. Microbiol.* 18, 715-727.

Bajaj, V., Lucas, R. L., Hwang, C. & Lee, C. A. (1996). Co-ordinate regulation of Salmonella typhimurium invasion genes by environmental and regulatory factors is mediated by control of hilA expression *Mol. Microbiol.* 22, 703-714.

Baker, C. S., Eöry, L. A., Yakhnin, H., Mercante, J., Romeo, T. & Babitzke, P. (2007). CsrA inhibits translation initiation of Escherichia coli hfq by binding to a single site overlapping the Shine-Dalgarno sequence *J. Bacteriol.* 189, 5472-5481.

Balbontín, R., Rowley, G., Pucciarelli, M. G., López-Garrido, J., Wormstone, Y., Lucchini, S., García-Del Portillo, F., Hinton, J. C. D. & Casadesús, J. (2006). DNA adenine methylation regulates virulence gene expression in Salmonella enterica serovar Typhimurium *J. Bacteriol.* 188, 8160-8168.

Baxter, M. A., Fahlen, T. F., Wilson, R. L. & Jones, B. D. (2003). HilE interacts with HilD and negatively regulates hilA transcription and expression of the Salmonella enterica serovar Typhimurium invasive phenotype *Infect. Immun.* 71, 1295-1305.

Bayliss, C. D. (2009). Determinants of phase variation rate and the fitness implications of differing rates for bacterial pathogens and commensals *FEMS Microbiol. Rev.* 33, 504-520.

Bäumler, A. J. & Fang, F. C. (2013). Host specificity of bacterial pathogens *Cold Spring Harb Perspect Med* 3, a010041.

Bäumler, A. J., Tsolis, R. M. & Heffron, F. (1996). Contribution of fimbrial operons to attachment to and invasion of epithelial cell lines by Salmonella typhimurium *Infect. Immun.* 64, 1862-1865.

Bäumler, A. J., Tsolis, R. M., Ficht, T. A. & Adams, L. G. (1998). Evolution of host adaptation in Salmonella enterica *Infect. Immun.* 66, 4579-4587.

Bäumler, A. J., Winter, S. E., Thiennimitr, P. & Casadesús, J. (2011). Intestinal and chronic infections: Salmonella lifestyles in hostile environments *Environ Microbiol Rep* 3, 508-

517.

Beaumont, H. J. E., Gallie, J., Kost, C., Ferguson, G. C. & Rainey, P. B. (2009). Experimental evolution of bet hedging *Nature* 462, 90-93.

Bickle, T. A. & Krüger, D. H. (1993). Biology of DNA restriction *Microbiol. Rev.* 57, 434-450.

Boddicker, J. D., Knosp, B. M. & Jones, B. D. (2003). Transcription of the Salmonella invasion gene activator, hilA, requires HilD activation in the absence of negative regulators *J. Bacteriol.* 185, 525-533.

Boye, E., Løbner-Olesen, A. & Skarstad, K. (2000). Limiting DNA replication to once and only once *EMBO Rep.* 1, 479-483.

Brill, J. A., Quinlan-Walshe, C. & Gottesman, S. (1988). Fine-structure mapping and identification of two regulators of capsule synthesis in Escherichia coli K-12 *J. Bacteriol.* 170, 2599-2611.

Broadbent, S. E., Davies, M. R. & van der Woude, M. W. (2010). Phase variation controls expression of Salmonella lipopolysaccharide modification genes by a DNA methylation-dependent mechanism *Mol. Microbiol.* 77, 337-353.

Brombacher, E., Dorel, C., Zehnder, A. J. B. & Landini, P. (2003). The curli biosynthesis regulator CsgD co-ordinates the expression of both positive and negative determinants for biofilm formation in Escherichia coli *Microbiology (Reading, Engl.)* 149, 2847-2857.

Bustamante, V. H., Martínez, L. C., Santana, F. J., Knodler, L. A., Steele-Mortimer, O. & Puente, J. L. (2008). HilD-mediated transcriptional cross-talk between SPI-1 and SPI-2 *Proc. Natl. Acad. Sci. U.S.A.* 105, 14591-14596.

Camacho, E. M. & Casadesús, J. (2002). Conjugal transfer of the virulence plasmid of Salmonella enterica is regulated by the leucine-responsive regulatory protein and DNA adenine methylation *Mol. Microbiol.* 44, 1589-1598.

Camacho, E. M. & Casadesús, J. (2005). Regulation of traJ transcription in the Salmonella virulence plasmid by strand-specific DNA adenine hemimethylation *Mol. Microbiol.* 57, 1700-1718.

Campbell, J. L. & Kleckner, N. (1990). E. coli oriC and the dnaA gene promoter are sequestered from dam methyltransferase following the passage of the chromosomal replication fork *Cell* 62, 967-979.

Cano, D. A., Domínguez-Bernal, G., Tierrez, A., García-Del Portillo, F. & Casadesús, J. (2002). Regulation of capsule synthesis and cell motility in Salmonella enterica by the essential gene igaA *Genetics* 162, 1513-1523.

Carballès, F., Bertrand, C., Bouché, J. P. & Cam, K. (1999). Regulation of Escherichia coli cell division genes ftsA and ftsZ by the two-component system rcsC-rcsB *Mol. Microbiol.* 34, 442-450.

Casadesús, J. & Low, D. (2006). Epigenetic gene regulation in the bacterial world *Microbiol. Mol. Biol. Rev.* 70, 830-856.

Casadesús, J. & Low, D. A. (2013). Programmed heterogeneity: epigenetic mechanisms in bacteria *J. Biol. Chem.* 288, 13929-13935.

Chan, K., Baker, S., Kim, C. C., Detweiler, C. S., Dougan, G. & Falkow, S. (2003). Genomic comparison of Salmonella enterica serovars and Salmonella bongori by use of an S.

enterica serovar typhimurium DNA microarray *J. Bacteriol.* 185, 553-563.

Chan, R. K., Botstein, D., Watanabe, T. & Ogata, Y. (1972). Specialized transduction of tetracycline resistance by phage P22 in Salmonella typhimurium. II. Properties of a high-frequency-transducing lysate *Virology* 50, 883-898.

Cherepanov, P. P. & Wackernagel, W. (1995). Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant *Gene* 158, 9-14.

Chessa, D., Winter, M. G., Nuccio, S., Tükel, C. & Bäumler, A. J. (2008). RosE represses Std fimbrial expression in Salmonella enterica serotype Typhimurium *Mol. Microbiol.* 68, 573-587.

Chessa, D., Winter, M. G., Jakomin, M. & Bäumler, A. J. (2009). Salmonella enterica serotype Typhimurium Std fimbriae bind terminal alpha(1,2)fucose residues in the cecal mucosa *Mol. Microbiol.* 71, 864-875.

Chilcott, G. S. & Hughes, K. T. (2000). Coupling of flagellar gene expression to flagellar assembly in Salmonella enterica serovar typhimurium and Escherichia coli *Microbiol. Mol. Biol. Rev.* 64, 694-708.

Chubiz, J. E. C., Golubeva, Y. A., Lin, D., Miller, L. D. & Slauch, J. M. (2010). FliZ regulates expression of the Salmonella pathogenicity island 1 invasion locus by controlling HilD protein activity in Salmonella enterica serovar typhimurium *J. Bacteriol.* 192, 6261-6270.

Claret, L. & Hughes, C. (2000). Functions of the subunits in the FlhD(2)C(2) transcriptional master regulator of bacterial flagellum biogenesis and swarming *J. Mol. Biol.* 303, 467-478.

Clegg, S., Purcell, B. K. & Pruckler, J. (1987). Characterization of genes encoding type 1 fimbriae of Klebsiella pneumoniae, Salmonella typhimurium, and Serratia marcescens *Infect. Immun.* 55, 281-287.

Cornelis, G. R. & Van Gijsegem, F. (2000). Assembly and function of type III secretory systems *Annu. Rev. Microbiol.* 54, 735-774.

Costa, C. S. & Antón, D. N. (2001). Role of the ftsA1p promoter in the resistance of mucoid mutants of Salmonella enterica to mecillinam: characterization of a new type of mucoid mutant *FEMS Microbiol. Lett.* 200, 201-205.

Cota, I., Sánchez-Romero, M. A., Hernández, S. B., Pucciarelli, M. G., García-Del Portillo, F. & Casadesús, J. (2015). Epigenetic Control of Salmonella enterica O-Antigen Chain Length: A Tradeoff between Virulence and Bacteriophage Resistance *PLoS Genet.* 11, e1005667.

Cummings, L. A., Wilkerson, W. D., Bergsbaken, T. & Cookson, B. T. (2006). In vivo, fliC expression by Salmonella enterica serovar Typhimurium is heterogeneous, regulated by ClpX, and anatomically restricted *Mol. Microbiol.* 61, 795-809.

Danese, P. N., Pratt, L. A. & Kolter, R. (2000). Exopolysaccharide production is required for development of Escherichia coli K-12 biofilm architecture *J. Bacteriol.* 182, 3593-3596.

Darwin, K. H. & Miller, V. L. (1999). InvF is required for expression of genes encoding proteins secreted by the SPI1 type III secretion apparatus in Salmonella typhimurium *J. Bacteriol.* 181, 4949-4954.

Datsenko, K. A. & Wanner, B. L. (2000). One-step inactivation of chromosomal genes in

Escherichia coli K-12 using PCR products *Proc. Natl. Acad. Sci. U.S.A.* 97, 6640-6645.

Delgado, M. A., Mouslim, C. & Groisman, E. A. (2006). The PmrA/PmrB and RcsC/YojN/RcsB systems control expression of the Salmonella O-antigen chain length determinant *Mol. Microbiol.* 60, 39-50.

Diekmann, S. (1987). DNA methylation can enhance or induce DNA curvature *EMBO J.* 6, 4213-4217.

Duguid, J. P., Anderson, E. S. & Campbell, I. (1966). Fimbriae and adhesive properties in Salmonellae *J Pathol Bacteriol* 92, 107-138.

Ebel, W., Vaughn, G. J., Peters, H. K. & Trempy, J. E. (1997). Inactivation of mdoH leads to increased expression of colanic acid capsular polysaccharide in Escherichia coli *J. Bacteriol.* 179, 6858-6861.

Edgar, R., Domrachev, M. & Lash, A. E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository *Nucleic Acids Res.* 30, 207-210.

Ellermeier, C. & Slauch, J. (2006). The genus Salmonella. In *The prokaryotes*, pp. 123-158M. Dworkin, S. Falkow, R. E, K. Schleifer & S. E. Edited by. New York, USA.

Ellermeier, C. D. & Slauch, J. M. (2003). RtsA and RtsB coordinately regulate expression of the invasion and flagellar genes in Salmonella enterica serovar Typhimurium *J. Bacteriol.* 185, 5096-5108.

Ellermeier, C. D., Janakiraman, A. & Slauch, J. M. (2002). Construction of targeted single copy lac fusions using lambda Red and FLP-mediated site-specific recombination in bacteria *Gene* 290, 153-161.

Ellermeier, C. D., Ellermeier, J. R. & Slauch, J. M. (2005). HilD, HilC and RtsA constitute a feed forward loop that controls expression of the SPI1 type three secretion system regulator hilA in Salmonella enterica serovar Typhimurium *Mol. Microbiol.* 57, 691-705.

Ellermeier, J. R. & Slauch, J. M. (2007). Adaptation to the host environment: regulation of the SPI1 type III secretion system in Salmonella enterica serovar Typhimurium *Curr. Opin. Microbiol.* 10, 24-29.

Emmerth, M., Goebel, W., Miller, S. I. & Hueck, C. J. (1999). Genomic subtraction identifies Salmonella typhimurium prophages, F-related plasmid sequences, and a novel fimbrial operon, stf, which are absent in Salmonella typhi *J. Bacteriol.* 181, 5652-5661.

Engel, J. D. & Hippel, von, P. H. (1978). Effects of methylation on the stability of nucleic acid conformations. Studies at the polymer level *J. Biol. Chem.* 253, 927-934.

Espinosa Alfaro, E. (2015). *Transcriptional regulation in Salmonella enterica by the LysR-type factor LeuO.* (J. Casadesús). Doctoral Thesis. Universidad de Sevilla.

Fàbrega, A. & Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation *Clin. Microbiol. Rev.* 26, 308-341.

Finlay, B. B. & Brumell, J. H. (2000). Salmonella interactions with host cells: in vitro to in vivo *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 355, 623-631.

Firth, N., Ippen-Ihler, K. & Skurray, R. (1996). Structure and function of the F factor and mechanism of conjugation. In *Escherichia coli and Salmonella: Cellular and Molecular Biology.*, pp. 2377 – 2401F. C. Neidhardt, R. Curtiss, J. L. Ingraham, K. B. Low, E. C. C. Lin & B. Magasanik. Edited by. Washington, DC: American Society for Microbiology Press.

Fitzgerald, D. M., Bonocora, R. P. & Wade, J. T. (2015). Correction: Comprehensive Mapping of the Escherichia coli Flagellar Regulatory Network *PLoS Genet.* 11, e1005456.

Folkesson, A., Advani, A., Sukupolvi, S., Pfeifer, J. D., Normark, S. & Löfdahl, S. (1999). Multiple insertions of fimbrial operons correlate with the evolution of Salmonella serovars responsible for human disease *Mol. Microbiol.* 33, 612-622.

Foster, J. W. & Hall, H. K. (1990). Adaptive acidification tolerance response of Salmonella typhimurium *J. Bacteriol.* 172, 771-778.

Francez-Charlot, A., Laugel, B., Van Gemert, A., Dubarry, N., Wiorowski, F., Castanié-Cornet, M., Gutierrez, C. & Cam, K. (2003). RcsCDB His-Asp phosphorelay system negatively regulates the flhDC operon in Escherichia coli *Mol. Microbiol.* 49, 823-832.

Friedhoff, P., Thomas, E. & Pingoud, A. (2003). Tyr212: a key residue involved in strand discrimination by the DNA mismatch repair endonuclease MutH *J. Mol. Biol.* 325, 285-297.

Galán, J. E. (1999). Interaction of Salmonella with host cells through the centisome 63 type III secretion system *Curr. Opin. Microbiol.* 2, 46-50.

Galán, J. E. (2001). Salmonella interactions with host cells: type III secretion at work *Annu. Rev. Cell Dev. Biol.* 17, 53-86.

Galán, J. E. & Collmer, A. (1999). Type III secretion machines: bacterial devices for protein delivery into host cells *Science* 284, 1322-1328.

García-Del Portillo, F., Pucciarelli, M. G. & Casadesús, J. (1999). DNA adenine methylase mutants of Salmonella typhimurium show defects in protein secretion, cell invasion, and M cell cytotoxicity *Proc. Natl. Acad. Sci. U.S.A.* 96, 11578-11583.

García-Quintanilla, M. & Casadesús, J. (2011). Virulence plasmid interchange between strains ATCC 14028, LT2, and SL1344 of Salmonella enterica serovar Typhimurium *Plasmid* 65, 169-175.

García-Quintanilla, M., Prieto, A. I., Barnes, L., Ramos-Morales, F. & Casadesús, J. (2006). Bile-induced curing of the virulence plasmid in Salmonella enterica serovar Typhimurium *J. Bacteriol.* 188, 7963-7965.

García-Quintanilla, M., Ramos-Morales, F. & Casadesús, J. (2008). Conjugal transfer of the Salmonella enterica virulence plasmid in the mouse intestine *J. Bacteriol.* 190, 1922-1927.

Gerstel, U. & Römling, U. (2003). The csgD promoter, a control unit for biofilm formation in Salmonella typhimurium *Res. Microbiol.* 154, 659-667.

Ghigo, J. M. (2001). Natural conjugative plasmids induce bacterial biofilm development *Nature* 412, 442-445.

Gillen, K. L. & Hughes, K. T. (1991). Negative regulatory loci coupling flagellin synthesis to flagellar assembly in Salmonella typhimurium *J. Bacteriol.* 173, 2301-2310.

Grafen, A. (1999). Formal Darwinism, the individual-as-maximizing-agent analogy and bet-hedging In *Proceedings of the Royal Society 266*, pp. 799-803. Presented at the Proceedings of the Royal Society 266.

Grassl, G. A. & Finlay, B. B. (2008). Pathogenesis of enteric Salmonella infections *Curr. Opin. Gastroenterol.* 24, 22-26.

Grimont, P. & Weill, F. (2007, November 2). Antigenic formulae of the Salmonellae serovars., WHO Collab Cent Ref Res Salmonella.

Groisman, E. A. & Ochman, H. (1997). How Salmonella became a pathogen *Trends Microbiol.* 5, 343-349.

Grund, S. & Weber, A. (1988). A new type of fimbriae on Salmonella typhimurium *Zentralblatt Veterinarmedizin Reihe B* 35, 779-782.

Gubbins, M. J., Lau, I., Will, W. R., Manchak, J. M., Raivio, T. L. & Frost, L. S. (2002). The positive regulator, TraJ, of the Escherichia coli F plasmid is unstable in a cpxA* background *J. Bacteriol.* 184, 5781-5788.

Gulig, P. A., Danbara, H., Guiney, D. G., Lax, A. J., Norel, F. & Rhen, M. (1993). Molecular analysis of spv virulence genes of the Salmonella virulence plasmids *Mol. Microbiol.* 7, 825-830.

Gunn, J. S. (2000). Mechanisms of bacterial resistance and response to bile *Microbes Infect.* 2, 907-913.

Haagmans, W. & van der Woude, M. (2000). Phase variation of Ag43 in Escherichia coli: Dam-dependent methylation abrogates OxyR binding and OxyR-mediated repression of transcription *Mol. Microbiol.* 35, 877-887.

Han, J. S., Kang, S., Lee, H., Kim, H. K. & Hwang, D. S. (2003). Sequential binding of SeqA to paired hemi-methylated GATC sequences mediates formation of higher order complexes *J. Biol. Chem.* 278, 34983-34989.

Hanahan, D. (1983). Studies on transformation of Escherichia coli with plasmids *J. Mol. Biol.* 166, 557-580.

Hautefort, I., Proença, M. J. & Hinton, J. C. D. (2003). Single-copy green fluorescent protein gene fusions allow accurate measurement of Salmonella gene expression in vitro and during infection of mammalian cells *Appl. Environ. Microbiol.* 69, 7480-7491.

Henderson, I. R. & Owen, P. (1999). The major phase-variable outer membrane protein of Escherichia coli structurally resembles the immunoglobulin A1 protease class of exported protein and is regulated by a novel mechanism involving Dam and oxyR *J. Bacteriol.* 181, 2132-2141.

Henderson, I. R., Meehan, M. & Owen, P. (1997). Antigen 43, a phase-variable bipartite outer membrane protein, determines colony morphology and autoaggregation in Escherichia coli K-12 *FEMS Microbiol. Lett.* 149, 115-120.

Hensel, M. (2000). Salmonella pathogenicity island 2 *Mol. Microbiol.* 36, 1015-1023.

Hensel, M. (2004). Evolution of pathogenicity islands of Salmonella enterica *Int. J. Med. Microbiol.* 294, 95-102.

Hernday, A. D., Braaten, B. A. & Low, D. A. (2003). The mechanism by which DNA adenine methylase and PapI activate the pap epigenetic switch. 12, 947-957.

Hernday, A., Krabbe, M., Braaten, B. & Low, D. (2002). Self-perpetuating epigenetic pili switches in bacteria *Proc. Natl. Acad. Sci. U.S.A.* 99 Suppl 4, 16470-16476.

Hernday, A., Braaten, B. & Low, D. (2004). The intricate workings of a bacterial epigenetic switch *Adv. Exp. Med. Biol.* 547, 83-89.

Hoiseth, S. K. & Stocker, B. A. (1981). Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccines *Nature* 291, 238-239.

Hsieh, P. (2001). Molecular mechanisms of DNA mismatch repair *Mutat. Res.* 486, 71-87.

Humphries, A. D., Townsend, S. M., Kingsley, R. A., Nicholson, T. L., Tsolis, R. M. & Bäumler, A. J. (2001). Role of fimbriae as antigens and intestinal colonization factors of Salmonella serovars *FEMS Microbiol. Lett.* 201, 121-125.

Humphries, A. D., Raffatellu, M., Winter, S., Weening, E. H., Kingsley, R. A., Droleskey, R., Zhang, S., Figueiredo, J., Khare, S., et al. (2003). The use of flow cytometry to detect expression of subunits encoded by 11 Salmonella enterica serotype Typhimurium fimbrial operons *Mol. Microbiol.* 48, 1357-1376.

Ikebe, T., Iyoda, S. & Kutsukake, K. (1999). Promoter analysis of the class 2 flagellar operons of Salmonella *Genes Genet. Syst.* 74, 179-183.

Inoue, H., Nojima, H. & Okayama, H. (1990). High efficiency transformation of Escherichia coli with plasmids *Gene* 96, 23-28.

Jakomin, M., Chessa, D., Bäumler, A. J. & Casadesús, J. (2008). Regulation of the Salmonella enterica std fimbrial operon by DNA adenine methylation, SeqA, and HdfR *J. Bacteriol.* 190, 7406-7413.

Jerome, L. J., van Biesen, T. & Frost, L. S. (1999). Degradation of FinP antisense RNA from F-like plasmids: the RNA-binding protein, FinO, protects FinP from ribonuclease E *J. Mol. Biol.* 285, 1457-1473.

Ji, H., Jiang, H., Ma, W., Johnson, D. S., Myers, R. M. & Wong, W. H. (2008). An integrated software system for analyzing ChIP-chip and ChIP-seq data *Nat. Biotechnol.* 26, 1293-1300.

Jones, G. W., Rabert, D. K., Svinarich, D. M. & Whitfield, H. J. (1982). Association of adhesive, invasive, and virulent phenotypes of Salmonella typhimurium with autonomous 60-megadalton plasmids *Infect. Immun.* 38, 476-486.

Julio, S. M., Heithoff, D. M., Provenzano, D., Klose, K. E., Sinsheimer, R. L., Low, D. A. & Mahan, M. J. (2001). DNA adenine methylase is essential for viability and plays a role in the pathogenesis of Yersinia pseudotuberculosis and Vibrio cholerae *Infect. Immun.* 69, 7610-7615.

Kelly, B. G., Vespermann, A. & Bolton, D. J. (2009). The role of horizontal gene transfer in the evolution of selected foodborne bacterial pathogens *Food Chem. Toxicol.* 47, 951-968.

Kingsley, R. A., Weening, E. H., Keestra, A. M. & Bäumler, A. J. (2002). Population heterogeneity of Salmonella enterica serotype Typhimurium resulting from phase variation of the lpf operon in vitro and in vivo *J. Bacteriol.* 184, 2352-2359.

Ko, M. & Park, C. (2000). H-NS-Dependent regulation of flagellar synthesis is mediated by a LysR family protein *J. Bacteriol.* 182, 4670-4672.

Koraimann, G., Teferle, K., Markolin, G., Woger, W. & Högenauer, G. (1996). The FinOP repressor system of plasmid R1: analysis of the antisense RNA control of traJ expression and conjugative DNA transfer *Mol. Microbiol.* 21, 811-821.

Kubori, T., Matsushima, Y., Nakamura, D., Uralil, J., Lara-Tejero, M., Sukhan, A., Galán, J. E. & Aizawa, S. I. (1998). Supramolecular structure of the Salmonella typhimurium type III protein secretion system *Science* 280, 602-605.

Kullik, I., Toledano, M. B., Tartaglia, L. A. & Storz, G. (1995). Mutational analysis of the redox-sensitive transcriptional regulator OxyR: regions important for oxidation and

transcriptional activation *J. Bacteriol.* 177, 1275-1284.

Kutsukake, K. & Iino, T. (1994). Role of the FliA-FlgM regulatory system on the transcriptional control of the flagellar regulon and flagellar formation in Salmonella typhimurium *J. Bacteriol.* 176, 3598-3605.

Kutsukake, K., Ohya, Y. & Iino, T. (1990). Transcriptional analysis of the flagellar regulon of Salmonella typhimurium *J. Bacteriol.* 172, 741-747.

Kücherer, C., Lother, H., Kölling, R., Schauzu, M. A. & Messer, W. (1986). Regulation of transcription of the chromosomal dnaA gene of Escherichia coli *Mol. Gen. Genet.* 205, 115-121.

la Cruz, De, M. A., Pérez-Morales, D., Palacios, I. J., Fernández-Mora, M., Calva, E. & Bustamante, V. H. (2015). The two-component system CpxR/A represses the expression of Salmonella virulence genes by affecting the stability of the transcriptional regulator HilD *Front Microbiol* 6, 807.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4 *Nature* 227, 680-685.

Lahiri, A., Das, P. & Chakravortty, D. (2009). Salmonella Typhimurium: insight into the multi-faceted role of the LysR-type transcriptional regulators in Salmonella *Int. J. Biochem. Cell Biol.* 41, 2129-2133.

Lambert, G., Kussell, E. & Kussel, E. (2014). Memory and fitness optimization of bacteria under fluctuating environments *PLoS Genet.* 10, e1004556.

Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome *Genome Biol.* 10, R25.

LaRock, D. L., Chaudhary, A. & Miller, S. I. (2015). Salmonellae interactions with host processes *Nat. Rev. Microbiol.* 13, 191-205.

Latasa, C., García, B., Echeverz, M., Toledo-Arana, A., Valle, J., Campoy, S., García-Del Portillo, F., Solano, C. & Lasa, I. (2012). Salmonella biofilm development depends on the phosphorylation status of RcsB *J. Bacteriol.* 194, 3708-3722.

Lee, I. S., Lin, J., Hall, H. K., Bearson, B. & Foster, J. W. (1995). The stationary-phase sigma factor sigma S (RpoS) is required for a sustained acid tolerance response in virulent Salmonella typhimurium *Mol. Microbiol.* 17, 155-167.

Liu, X. & Matsumura, P. (1994). The FlhD/FlhC complex, a transcriptional activator of the Escherichia coli flagellar class II operons *J. Bacteriol.* 176, 7345-7351.

Loenen, W. A. M., Dryden, D. T. F., Raleigh, E. A., Wilson, G. G. & Murray, N. E. (2014). Highlights of the DNA cutters: a short history of the restriction enzymes *Nucleic Acids Res.* 42, 3-19.

Lostroh, C. P. & Lee, C. A. (2001). The Salmonella pathogenicity island-1 type III secretion system *Microbes Infect.* 3, 1281-1291.

Low, D. A. & Casadesús, J. (2008). Clocks and switches: bacterial gene regulation by DNA adenine methylation *Curr. Opin. Microbiol.* 11, 106-112.

Low, D. A., Weyand, N. J. & Mahan, M. J. (2001). Roles of DNA adenine methylation in regulating bacterial gene expression and virulence *Infect. Immun.* 69, 7197-7204.

López-Garrido, J. & Casadesús, J. (2010). Regulation of Salmonella enterica pathogenicity

island 1 by DNA adenine methylation *Genetics* 184, 637-649.

López-Garrido, J. & Casadesús, J. (2012). Crosstalk between virulence loci: regulation of Salmonella enterica pathogenicity island 1 (SPI-1) by products of the std fimbrial operon *PLoS ONE* 7, e30499.

López-Garrido, J., Puerta-Fernández, E. & Casadesús, J. (2014). A eukaryotic-like 3' untranslated region in Salmonella enterica hilD mRNA *Nucleic Acids Res.* 42, 5894-5906.

Lutz, R. & Bujard, H. (1997). Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements *Nucleic Acids Res.* 25, 1203-1210.

Løbner-Olesen, A., Skovgaard, O. & Marinus, M. G. (2005). Dam methylation: coordinating cellular processes *Curr. Opin. Microbiol.* 8, 154-160.

Macián, F., Pérez-Roger, I. & Armengod, M. E. (1994). An improved vector system for constructing transcriptional lacZ fusions: analysis of regulation of the dnaA, dnaN, recF and gyrB genes of Escherichia coli *Gene* 145, 17-24.

Macnab, R. M. (2003). How bacteria assemble flagella *Annu. Rev. Microbiol.* 57, 77-100.

Maddocks, S. E. & Oyston, P. C. F. (2008). Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins *Microbiology (Reading, Engl.)* 154, 3609-3623.

Majdalani, N. & Gottesman, S. (2005). The Rcs phosphorelay: a complex signal transduction system *Annu. Rev. Microbiol.* 59, 379-405.

Marinus, M. (1996). Methylation of DNA. In *Escherichia coli and Salmonella: cellular and molecular biology*, pp. 782-791F. E. al. Ed. Washington, DC.

Marinus, M. G. & Casadesús, J. (2009). Roles of DNA adenine methylation in host-pathogen interactions: mismatch repair, transcriptional regulation, and more *FEMS Microbiol. Rev.* 33, 488-503.

Marinus, M. G., Poteete, A. & Arraj, J. A. (1984). Correlation of DNA adenine methylase activity with spontaneous mutability in Escherichia coli K-12 *Gene* 28, 123-125.

May, T. & Okabe, S. (2008). Escherichia coli harboring a natural IncF conjugative F plasmid develops complex mature biofilms by stimulating synthesis of colanic acid and Curli *J. Bacteriol.* 190, 7479-7490.

McClelland, M., Sanderson, K. E., Spieth, J., Clifton, S. W., Latreille, P., Courtney, L., Porwollik, S., Ali, J., Dante, M., et al. (2001). Complete genome sequence of Salmonella enterica serovar Typhimurium LT2 *Nature* 413, 852-856.

McQuiston, J. R., Parrenas, R., Ortiz-Rivera, M., Gheesling, L., Brenner, F. & Fields, P. I. (2004). Sequencing and comparative analysis of flagellin genes fliC, fljB, and flpA from Salmonella *J. Clin. Microbiol.* 42, 1923-1932.

McQuiston, J. R., Herrera-Leon, S., Wertheim, B. C., Doyle, J., Fields, P. I., Tauxe, R. V. & Logsdon, J. M. (2008). Molecular phylogeny of the salmonellae: relationships among Salmonella species and subspecies determined from four housekeeping genes and evidence of lateral gene transfer events *J. Bacteriol.* 190, 7060-7067.

Messer, W. & Noyer-Weidner, M. (1988). Timing and targeting: the biological functions of Dam methylation in E. coli *Cell* 54, 735-737.

Miller, J. H. (1972). *Experiments in Molecular Genetics*. *Cold Spring Harbor Laboratory Press*. Cold Spring Harbor, N.Y.

Minamino, T. & Imada, K. (2015). The bacterial flagellar motor and its structural diversity *Trends Microbiol.* 23, 267-274.

Moest, T. P. & Méresse, S. (2013). Salmonella T3SSs: successful mission of the secret(ion) agents *Curr. Opin. Microbiol.* 16, 38-44.

Morrow, B. J., Graham, J. E. & Curtiss, R. (1999). Genomic subtractive hybridization and selective capture of transcribed sequences identify a novel Salmonella typhimurium fimbrial operon and putative transcriptional regulator that are absent from the Salmonella typhi genome *Infect. Immun.* 67, 5106-5116.

Mouslim, C. & Groisman, E. A. (2003). Control of the Salmonella ugd gene by three two-component regulatory systems *Mol. Microbiol.* 47, 335-344.

Mouslim, C., Latifi, T. & Groisman, E. A. (2003). Signal-dependent requirement for the co-activator protein RcsA in transcription of the RcsB-regulated ugd gene *J. Biol. Chem.* 278, 50588-50595.

Mouslim, C., Delgado, M. & Groisman, E. A. (2004). Activation of the RcsC/YojN/RcsB phosphorelay system attenuates Salmonella virulence *Mol. Microbiol.* 54, 386-395.

Moxon, E. R., Rainey, P. B., Nowak, M. A. & Lenski, R. E. (1994). Adaptive evolution of highly mutable loci in pathogenic bacteria *Curr. Biol.* 4, 24-33.

Moxon, R., Bayliss, C. & Hood, D. (2006). Bacterial contingency loci: the role of simple sequence DNA repeats in bacterial adaptation *Annu. Rev. Genet.* 40, 307-333.

Nicholson, B. & Low, D. (2000). DNA methylation-dependent regulation of pef expression in Salmonella typhimurium *Mol. Microbiol.* 35, 728-742.

Norris, T. L., Kingsley, R. A. & Bäumler, A. J. (1998). Expression and transcriptional control of the Salmonella typhimurium Ipf fimbrial operon by phase variation *Mol. Microbiol.* 29, 311-320.

Nuccio, S. & Bäumler, A. J. (2007). Evolution of the chaperone/usher assembly pathway: fimbrial classification goes Greek *Microbiol. Mol. Biol. Rev.* 71, 551-575.

Nunes, J. S., Lawhon, S. D., Rossetti, C. A., Khare, S., Figueiredo, J. F., Gull, T., Burghardt, R. C., Bäumler, A. J., Tsolis, R. M., et al. (2010). Morphologic and cytokine profile characterization of Salmonella enterica serovar typhimurium infection in calves with bovine leukocyte adhesion deficiency *Vet. Pathol.* 47, 322-333.

Ochman, H. & Wilson, A. C. (1987). Evolution in bacteria: evidence for a universal substitution rate in cellular genomes *J. Mol. Evol.* 26, 74-86.

Ochman, H., Soncini, F. C., Solomon, F. & Groisman, E. A. (1996). Identification of a pathogenicity island required for Salmonella survival in host cells *Proc. Natl. Acad. Sci. U.S.A.* 93, 7800-7804.

Olekhnovich, I. N. & Kadner, R. J. (2002). DNA-binding activities of the HilC and HilD virulence regulatory proteins of Salmonella enterica serovar Typhimurium *J. Bacteriol.* 184, 4148-4160.

Olsén, A., Jonsson, A. & Normark, S. (1989). Fibronectin binding mediated by a novel class of surface organelles on Escherichia coli *Nature* 338, 652-655.

Oshima, T., Wada, C., Kawagoe, Y., Ara, T., Maeda, M., Masuda, Y., Hiraga, S. & Mori, H.

(2002). Genome-wide analysis of deoxyadenosine methyltransferase-mediated control of gene expression in Escherichia coli *Mol. Microbiol.* 45, 673-695.

Ou, J. T., Baron, L. S., Dai, X. Y. & Life, C. A. (1990). The virulence plasmids of Salmonella serovars typhimurium, choleraesuis, dublin, and enteritidis, and the cryptic plasmids of Salmonella serovars copenhagen and sendai belong to the same incompatibility group, but not those of Salmonella serovars durban, gallinarum, give, infantis and pullorum *Microb. Pathog.* 8, 101-107.

Paytubi, S., Aznar, S., Madrid, C., Balsalobre, C., Dillon, S. C., Dorman, C. J. & Juárez, A. (2014). A novel role for antibiotic resistance plasmids in facilitating Salmonella adaptation to non-host environments *Environ. Microbiol.* 16, 950-962.

Pescaretti, M. D. L. M., Morero, R. & Delgado, M. A. (2009). Identification of a new promoter for the response regulator rcsB expression in Salmonella enterica serovar Typhimurium *FEMS Microbiol. Lett.* 300, 165-173.

Peterson, S. N. & Reich, N. O. (2006). GATC flanking sequences regulate Dam activity: evidence for how Dam specificity may influence pap expression *J. Mol. Biol.* 355, 459-472.

Petrone, B. L., Stringer, A. M. & Wade, J. T. (2014). Identification of HilD-regulated genes in Salmonella enterica serovar Typhimurium *J. Bacteriol.* 196, 1094-1101.

Popoff, M. Y., Bockemühl, J. & Gheesling, L. L. (2004). Supplement 2002 (no. 46) to the Kauffmann-White scheme *Res. Microbiol.* 155, 568-570.

Porwollik, S. & McClelland, M. (2003). Lateral gene transfer in Salmonella *Microbes Infect.* 5, 977-989.

Porwollik, S., Wong, R. M. & McClelland, M. (2002). Evolutionary genomics of Salmonella: gene acquisitions revealed by microarray analysis *Proc. Natl. Acad. Sci. U.S.A.* 99, 8956-8961.

Porwollik, S., Boyd, E. F., Choy, C., Cheng, P., Florea, L., Proctor, E. & McClelland, M. (2004). Characterization of Salmonella enterica subspecies I genovars by use of microarrays *J. Bacteriol.* 186, 5883-5898.

Prieto, A. I., Ramos-Morales, F. & Casadesús, J. (2004). Bile-induced DNA damage in Salmonella enterica *Genetics* 168, 1787-1794.

Prigent-Combaret, C., Brombacher, E., Vidal, O., Ambert, A., Lejeune, P., Landini, P. & Dorel, C. (2001). Complex regulatory network controls initial adhesion and biofilm formation in Escherichia coli via regulation of the csgD gene *J. Bacteriol.* 183, 7213-7223.

Pucciarelli, M. G., Prieto, A. I., Casadesús, J. & García-Del Portillo, F. (2002). Envelope instability in DNA adenine methylase mutants of Salmonella enterica *Microbiology (Reading, Engl.)* 148, 1171-1182.

Que, F., Wu, S. & Huang, R. (2013). Salmonella pathogenicity island 1(SPI-1) at work *Curr. Microbiol.* 66, 582-587.

Quinlan, A. R. & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features *Bioinformatics* 26, 841-842.

Rotger, R. & Casadesús, J. (1999). The virulence plasmids of Salmonella *Int. Microbiol.* 2, 177-184.

Römling, U., Bian, Z., Hammar, M., Sierralta, W. D. & Normark, S. (1998). Curli fibers are highly conserved between Salmonella typhimurium and Escherichia coli with respect to operon structure and regulation *J. Bacteriol.* 180, 722-731.

Saini, S. & Rao, C. V. (2010). SprB is the molecular link between Salmonella pathogenicity island 1 (SPI1) and SPI4 *J. Bacteriol.* 192, 2459-2462.

Saini, S., Brown, J. D., Aldridge, P. D. & Rao, C. V. (2008). FliZ Is a posttranslational activator of FlhD4C2-dependent flagellar gene expression *J. Bacteriol.* 190, 4979-4988.

Saini, S., Pearl, J. A. & Rao, C. V. (2009). Role of FimW, FimY, and FimZ in regulating the expression of type i fimbriae in Salmonella enterica serovar Typhimurium *J. Bacteriol.* 191, 3003-3010.

Saini, S., Slauch, J. M., Aldridge, P. D. & Rao, C. V. (2010). Role of cross talk in regulating the dynamic expression of the flagellar Salmonella pathogenicity island 1 and type 1 fimbrial genes *J. Bacteriol.* 192, 5767-5777.

Sanderson, K. E., Kadam, S. K. & MacLachlan, P. R. (1983). Derepression of F factor function in Salmonella typhimurium *Can. J. Microbiol.* 29, 1205-1212.

Sansonetti, P. J. (2004). War and peace at mucosal surfaces *Nat. Rev. Immunol.* 4, 953-964.

Santos, R. L., Zhang, S., Tsolis, R. M., Kingsley, R. A., Adams, L. G. & Bäumler, A. J. (2001a). Animal models of Salmonella infections: enteritis versus typhoid fever *Microbes Infect.* 3, 1335-1344.

Santos, R. L., Tsolis, R. M., Zhang, S., Ficht, T. A., Bäumler, A. J. & Adams, L. G. (2001b). Salmonella-induced cell death is not required for enteritis in calves *Infect. Immun.* 69, 4610-4617.

Schechter, L. M. & Lee, C. A. (2001). AraC/XylS family members, HilC and HilD, directly bind and derepress the Salmonella typhimurium hilA promoter *Mol. Microbiol.* 40, 1289-1299.

Schell, M. A. (1993). Molecular biology of the LysR family of transcriptional regulators *Annu. Rev. Microbiol.* 47, 597-626.

Schmieger, H. (1972). Phage P22-mutants with increased or decreased transduction abilities *Mol. Gen. Genet.* 119, 75-88.

Shippy, D. C., Eakley, N. M., Mikheil, D. M. & Fadl, A. A. (2013). Role of StdA in adhesion of Salmonella enterica serovar Enteritidis phage type 8 to host intestinal epithelial cells *Gut Pathog* 5, 43.

Silverman, M., Zieg, J., Hilmen, M. & Simon, M. (1979). Phase variation in Salmonella: genetic analysis of a recombinational switch *Proc. Natl. Acad. Sci. U.S.A.* 76, 391-395.

Silverman, P. M., Wickersham, E. & Harris, R. (1991). Regulation of the F plasmid traY promoter in Escherichia coli by host and plasmid factors *J. Mol. Biol.* 218, 119-128.

Singer, H. M., Kühne, C., Deditius, J. A., Hughes, K. T. & Erhardt, M. (2014). The Salmonella Spi1 virulence regulatory protein HilD directly activates transcription of the flagellar master operon flhDC *J. Bacteriol.* 196, 1448-1457.

Smith, C., Stringer, A. M., Mao, C., Palumbo, M. J. & Wade, J. T. (2016). Mapping the Regulatory Network for Salmonella enterica Serovar Typhimurium Invasion *MBio* 7.

SMITH, H. O. & H, L. (1964). Two Sequential Repressions of Dna Synthesis in the Estalishment of Lysogeny by Phage 22 and Its Mutants *Proc. Natl. Acad. Sci. U.S.A.* 52,

356-363.

Soto, G. E. & Hultgren, S. J. (1999). Bacterial adhesins: common themes and variations in architecture and assembly *J. Bacteriol.* 181, 1059-1071.

Starcic-Erjavec, M., van Putten, J. P. M., Gaastra, W., Jordi, B. J. A. M., Grabnar, M. & Zgur-Bertok, D. (2003). H-NS and Lrp serve as positive modulators of traJ expression from the Escherichia coli plasmid pRK100 *Mol. Genet. Genomics* 270, 94-102.

Stolpe, H., Grund, S. & Schröder, W. (1994). Purification and partial characterization of type 3 fimbriae from Salmonella typhimurium var. copenhagen *Zentralbl. Bakteriol.* 281, 8-15.

Stout, V. & Gottesman, S. (1990). RcsB and RcsC: a two-component regulator of capsule synthesis in Escherichia coli *J. Bacteriol.* 172, 659-669.

Taghbalout, A., Landoulsi, A., Kern, R., Yamazoe, M., Hiraga, S., Holland, B., Kohiyama, M. & Malki, A. (2000). Competition between the replication initiator DnaA and the sequestration factor SeqA for binding to the hemimethylated chromosomal origin of E. coli in vitro *Genes Cells* 5, 873-884.

Tennant, S. M., Hartland, E. L., Phumoonna, T., Lyras, D., Rood, J. I., Robins-Browne, R. M. & van Driel, I. R. (2008). Influence of gastric acid on susceptibility to infection with ingested bacterial pathogens *Infect. Immun.* 76, 639-645.

Teplitski, M., Goodier, R. I. & Ahmer, B. M. M. (2003). Pathways leading from BarA/SirA to motility and virulence gene expression in Salmonella *J. Bacteriol.* 185, 7257-7265.

Thorvaldsdóttir, H., Robinson, J. T. & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration *Brief. Bioinformatics* 14, 178-192.

Tindall, B. J., Grimont, P. A. D., Garrity, G. M. & Euzéby, J. P. (2005). Nomenclature and taxonomy of the genus Salmonella *Int. J. Syst. Evol. Microbiol.* 55, 521-524.

Tinker, J. K. & Clegg, S. (2000). Characterization of FimY as a coactivator of type 1 fimbrial expression in Salmonella enterica serovar Typhimurium *Infect. Immun.* 68, 3305-3313.

Torreblanca, J. & Casadesús, J. (1996). DNA adenine methylase mutants of Salmonella typhimurium and a novel dam-regulated locus *Genetics* 144, 15-26.

Torreblanca, J., Marqués, S. & Casadesús, J. (1999). Synthesis of FinP RNA by plasmids F and pSLT is regulated by DNA adenine methylation *Genetics* 152, 31-45.

Townsend, S. M., Kramer, N. E., Edwards, R., Baker, S., Hamlin, N., Simmonds, M., Stevens, K., Maloy, S., Parkhill, J., et al. (2001). Salmonella enterica serovar Typhi possesses a unique repertoire of fimbrial gene sequences *Infect. Immun.* 69, 2894-2901.

Tsolis, R. M., Adams, L. G., Ficht, T. A. & Bäumler, A. J. (1999). Contribution of Salmonella typhimurium virulence factors to diarrheal disease in calves *Infect. Immun.* 67, 4879-4885.

Turner, K. H., Vallet-Gely, I. & Dove, S. L. (2009). Epigenetic control of virulence gene expression in Pseudomonas aeruginosa by a LysR-type transcription regulator *PLoS Genet.* 5, e1000779.

Urig, S., Gowher, H., Hermann, A., Beck, C., Fatemi, M., Humeny, A. & Jeltsch, A. (2002). The Escherichia coli dam DNA methyltransferase modifies DNA in a highly processive reaction *J. Mol. Biol.* 319, 1085-1096.

van der Velden, A. W., Bäumler, A. J., Tsolis, R. M. & Heffron, F. (1998). Multiple fimbrial adhesins are required for full virulence of Salmonella typhimurium in mice *Infect. Immun.* 66, 2803-2808.

van der Woude, M. W. (2006). Re-examining the role and random nature of phase variation *FEMS Microbiol. Lett.* 254, 190-197.

van der Woude, M. W. (2011). Phase variation: how to create and coordinate population diversity *Curr. Opin. Microbiol.* 14, 205-211.

van der Woude, M. W. & Bäumler, A. J. (2004). Phase and antigenic variation in bacteria *Clin. Microbiol. Rev.* 17, 581-611, table of contents.

Veening, J., Smits, W. K. & Kuipers, O. P. (2008). Bistability, epigenetics, and bet-hedging in bacteria *Annu. Rev. Microbiol.* 62, 193-210.

Velge, P., Wiedemann, A., Rosselin, M., Abed, N., Boumart, Z., Chaussé, A. M., Grépinet, O., Namdari, F., Roche, S. M., et al. (2012). Multiplicity of Salmonella entry mechanisms, a new paradigm for Salmonella pathogenesis *Microbiologyopen* 1, 243-258.

Wagner, C. & Hensel, M. (2011). Adhesive mechanisms of Salmonella enterica *Adv. Exp. Med. Biol.* 715, 17-34.

Waldron, D. E., Owen, P. & Dorman, C. J. (2002). Competitive interaction of the OxyR DNA-binding protein and the Dam methylase at the antigen 43 gene regulatory region in Escherichia coli *Mol. Microbiol.* 44, 509-520.

Wallecha, A., Munster, V., Correnti, J., Chan, T. & van der Woude, M. (2002). Dam- and OxyR-dependent phase variation of agn43: essential elements and evidence for a new role of DNA methylation *J. Bacteriol.* 184, 3338-3347.

Watson, K. G. & Holden, D. W. (2010). Dynamics of growth and dissemination of Salmonella in vivo *Cell. Microbiol.* 12, 1389-1397.

Weening, E. H., Barker, J. D., Laarakker, M. C., Humphries, A. D., Tsolis, R. M. & Bäumler, A. J. (2005). The Salmonella enterica serotype Typhimurium lpf, bcf, stb, stc, std, and sth fimbrial operons are required for intestinal persistence in mice *Infect. Immun.* 73, 3358-3366.

Wehland, M. & Bernhard, F. (2000). The RcsAB box. Characterization of a new operator essential for the regulation of exopolysaccharide biosynthesis in enteric bacteria *J. Biol. Chem.* 275, 7013-7020.

Will, W. R., Lu, J. & Frost, L. S. (2004). The role of H-NS in silencing F transfer gene expression during entry into stationary phase *Mol. Microbiol.* 54, 769-782.

Wion, D. & Casadesús, J. (2006). N6-methyl-adenine: an epigenetic signal for DNA-protein interactions *Nat. Rev. Microbiol.* 4, 183-192.

Wolf, D. M., Vazirani, V. V. & Arkin, A. P. (2005). Diversity in times of adversity: probabilistic strategies in microbial survival games *J. Theor. Biol.* 234, 227-253.

Wolfgang, M., van Putten, J. P., Hayes, S. F., Dorward, D. & Koomey, M. (2000). Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili *EMBO J.* 19, 6408-6418.

Yeh, K. S., Hancox, L. S. & Clegg, S. (1995). Construction and characterization of a fimZ mutant of Salmonella typhimurium *J. Bacteriol.* 177, 6861-6865.

Zaim, J. & Kierzek, A. M. (2003). The structure of full-length LysR-type transcriptional

regulators. Modeling of the full-length OxyR transcription factor dimer *Nucleic Acids Res.* 31, 1444-1454.

Zatyka, M. & Thomas, C. M. (1998). Control of genes for conjugative transfer of plasmids and other mobile elements *FEMS Microbiol. Rev.* 21, 291-319.

Zhang, X. L., Morris, C. & Hackett, J. (1997). Molecular cloning, nucleotide sequence, and function of a site-specific recombinase encoded in the major "pathogenicity island" of Salmonella typhi *Gene* 202, 139-146.

Zhang, X. L., Tsui, I. S., Yip, C. M., Fung, A. W., Wong, D. K., Dai, X., Yang, Y., Hackett, J. & Morris, C. (2000). Salmonella enterica serovar typhi uses type IVB pili to enter human intestinal epithelial cells *Infect. Immun.* 68, 3067-3073.

Zhou, D. & Galán, J. (2001). Salmonella entry into host cells: the work in concert of type III secreted effector proteins *Microbes Infect.* 3, 1293-1298.

Zyskind, J. W. & Smith, D. W. (1992). DNA replication, the bacterial cell cycle, and cell growth *Cell* 69, 5-8.

SUPPLEMENTARY DATA

Table SM. 1 Microarray results

GENES UP-REGULATED BY *stdEF* OVEREXPRESSION

STM	SL	Gene	fold change StdEF vs ΔstdEF (1)	fold change StdEF vs ΔstdEF (2)	Description
			VIRULENCE		
1043	SL0982		6,4	6,1	attachment/invasion protein
			CONJUGATION		
	SLP1_0027	*TraP*	2,8	2,6	conjugative transfer protein
	SLP1_0028	*TraB*	2,1	1,1	conjugative transfer: assembly
	SLP1_0030	*TraE*	3,7	3,1	conjugative transfer: assembly
	SLP1_0031	*TraL*	3,8	3,1	conjugative transfer: assembly
	SLP1_0032	*TraA*	4,3	4,9	conjugative transfer: fimbrial subunit
	SLP1_0033	*TraY*	4,0	4,0	conjugative transfer: oriT nicking
			METABOLISM		
191	SL0192	fhuA	3,8	3,4	ferrichrome outer membrane transporter
1343	SL1277	nlpC	3,3	3,5	lipoprotein
1344	SL1278	ydiV	2,5	1,3	hypothetical protein
3024	SL3002	yohM	2,1	1,6	nickel/cobalt efflux protein RcnA
3025	SL3003	stdE	10,3	10,0	
30251N	SL3004	stdF	10,6	10,5	
3128	SL3102	ordL	3,9	3,7	putative oxidoreductase
			OTHERS		
509	SL0502		2,4	1,8	putative outer membrane protein
3112	SL3096		2,6	1,9	murein transglycosylase C
3123	SL3097		3,2	2,4	putative arylsulfatase regulator
3125	SL3099		2,8	1,5	putative cytoplasmic protein
3126	SL3100		2,7	2,4	putative amino acid transporter
3127	SL3101		3,0	3,1	putative cytoplasmic protein
3129	SL3103		4,2	3,3	putative NAD-dependent aldehyde dehydrogenase
3169	SL3143		3,1	2,6	putative periplasmic dicarboxylate-binding protein
3170	SL3144		2,6	2,9	putative inner membrane protein
3171	SL3145	*ygiK*	2,2	2,5	putative transporter
3688	SL3653		2,3	2,3	putative cytoplasmic protein
3805	SL3773	*yidH*	2,1	1,0	putative inner membrane protein
3897	SL3858	*yifA*	3,2	2,9	transcriptional regulator HdfR
4548	SL4479	*bglJ*	2,5	1,4	DNA-binding transcriptional activator BglJ
4549	SL4480		3,0	2,2	putative cytoplasmic protein
	SLP2_0078		2,2	1,5	
	SLP2_0079		2,9	1,7	

GENES DOWN-REGULATED BY *stdEF* OVEREXPRESSION

STM	SL	gene	fold change StdEF/ ΔstdEF (1)	fold change StdEF/Δstd EF (2)	Description
			VIRULENCE		
1088	SL1027	*pipB*	-2,35261	-2,79374	secreted effector protein
1090	SL1029	*pipC*	-7,23594	-6,81151	pathogenicity island-encoded protein C
1091	SL1030	*sopB*	-8,07052	-8,51238	secreted effector protein
1593	SL1524	*srfA*	-4,63622	-4,43075	putative virulence protein
1594	SL1525	*srfB*	-3,99769	-4,23264	putative virulence protein
1595	SL1526	*srfC*	-4,13812	-3,67496	putative virulence protein
1855	SL1784	*sopE2*	-3,19064	-3,38909	type III-secreted effector protein
2066	SL2043	*sopA*	-4,45486	-4,58496	secreted effector protein
2865	SL2845	*avrA*	-2,9673	-2,92411	secreted effector protein
2866	SL2846	*sprB*	-4,30304	-3,2128	transcriptional regulator
2867	SL2847	*hilC*	-4,90612	-4,12935	invasion regulatory protein
2868	SL2848	*orgC*	-3,76945	-3,71423	putative cytoplasmic protein
2869	SL2849	*orgB*	-2,49114	-2,97335	needle complex export protein
2870	SL2850	*orgA*	-6,7807	-6,65603	needle complex assembly protein
2871	SL2851	*prgK*	-7,63394	-7,46371	needle complex inner membrane lipoprotein
2872	SL2852	*prgJ*	-8,37266	-8,47918	needle complex minor subunit
2873	SL2853	*prgI*	-8,53071	-7,79621	needle complex major subunit
2874	SL2854	*prgH*	-4,87411	-5,62474	needle complex inner membrane protein
2875	SL2855	*hilD*	-4,34678	-4,29323	invasion protein regulatory protein
2876	SL2856	*hilA*	-7,13183	-7,21907	invasion protein regulator
2877	SL2857	*iagB*	-6,66318	-5,83615	invasion protein precursor
2882	SL2861	*sipA*	-6,38297	-7,45432	secreted effector protein
2883	SL2862	*sipD*	-8,10815	-7,96644	translocation machinery component
2884	SL2863	*sipC*	-6,75384	-6,93525	translocation machinery component
2885	SL2864	*sipB*	-6,98067	-6,70203	translocation machinery component
2886	SL2865	*sicA*	-6,11606	-6,65031	secretion chaperone
2887	SL2866	*spaS*	-2,24243	-2,27852	surface presentation of antigens protein SpaS
2888	SL2867	*spaR*	-3,70299	-3,50458	needle complex export protein
2889	SL2868	*spaQ*	-4,65043	-3,48878	needle complex export protein
2890	SL2869	*spaP*	-4,84355	-3,47487	surface presentation of antigens protein SpaP
2891	SL2870	*spaQ*	-7,1891	-6,74103	surface presentation of antigens protein SpaO
2892	SL2871	*invJ*	-7,73751	-6,92514	needle length control protein
2893	SL2872	*invI*	-6,50147	-6,5762	needle complex assembly protein
2894	SL2873	*invC*	-6,54299	-6,27668	ATP synthase SpaL
2895	SL2874	*invB*	-7,80109	-7,30746	secretion chaperone
2896	SL2875	*invA*	-6,29927	-6,23907	needle complex export protein

2897	SL2876	*invE*	-5,15772	-4,42613	invasion protein
2898	SL2877	*invG*	-7,36066	-7,40968	outer membrane secretin precursor
2899	SL2878	*invF*	-9,005	-8,25772	invasion regulatory protein
2900	SL2879	*invH*	-5,75796	-5,85947	needle complex outer membrane lipoprotein precursor
2945	SL2924	*sopD*	-6,04938	-5,80587	secreted effector protein
4257	SL4193	*siiA*	-4,86275	-3,97478	
4258	SL4194	*siiB*	-5,64156	-5,12549	
4259	SL4195	*siiC*	-4,48573	-4,22586	
4260	SL4196	*siiD*	-6,2381	-5,52023	
4261	SL4197	*siiE*	-5,55131	-6,32293	
4262	SL4198	*siiF*	-3,85186	-3,66006	
			FLAGELLUM		
1171	SL1108	*flgG*	-5,71429	-5,21887	flagellar basal body rod protein FlgG
1172	SL1109	*flgM*	-3,9965	-4,33879	anti-sigma28 factor FlgM
1173	SL1110	*flgA*	-3,46902	-3,68834	flagellar basal body P-ring biosynthesis protein FlgA
1174	SL1111	*flgB*	-7,63659	-7,1108	flagellar basal body rod protein FlgB
1175	SL1112	*flgC*	-9,17839	-8,18843	flagellar basal body rod protein FlgC
1176	SL1113	*flgD*	-8,29584	-8,09363	flagellar basal body rod modification protein
1177	SL1114	*flgE*	-8,10062	-7,37697	flagellar hook protein FlgE
1178	SL1115	*flgF*	-7,55285	-6,77852	flagellar basal body rod protein FlgF
1179	SL1116	*flgG*	-7,06958	-6,1296	flagellar basal body rod protein FlgG
1180	SL1117	*flgH*	-5,68632	-5,07144	flagellar basal body L-ring protein
1181	SL1118	*flgI*	-5,76343	-5,37619	flagellar basal body P-ring protein
1182	SL1119	*flgJ*	-6,11105	-5,34008	peptidoglycan hydrolase
1183	SL1120	*flgK*	-7,62081	-7,72774	flagellar hook-associated protein FlgK
1184	SL1121	*flgL*	-6,80263	-6,42102	flagellar hook-associated protein FlgL
1912	SL1847	*flhE*	-5,17649	-4,25584	flagellar protein
1913	SL1848	*flha*	-4,43008	-3,93142	flagellar biosynthesis protein FlhA
1914	SL1849	*flhB*	-2,60218	-2,42441	flagellar biosynthesis protein FlhB
1912	SL1847	*flhE*	-5,17649	-4,25584	flagellar protein
1913	SL1848	*flha*	-4,43008	-3,93142	flagellar biosynthesis protein FlhA
1914	SL1849	*flhB*	-2,60218	-2,42441	flagellar biosynthesis protein FlhB
1915	SL1850	*flhZ*	-6,96302	-5,87415	
1916	SL1851	*flhY*	-7,81919	-6,54635	
1922	SL1857	*motB*	-7,33594	-7,39911	flagellar motor protein MotB
1923	SL1858	*motA*	-7,02063	-7,2536	flagellar motor protein MotA
1924	SL1859	*flhC*	-4,19042	-4,08165	transcriptional activator FlhC
1925	SL1860	*flhD*	-3,70422	-4,19309	transcriptional activator FlhD
1955	SL1884	*fliZ*	-5,94152	-6,62262	protein FliZ
1956	SL1885	*fliA*	-7,98073	-8,08624	flagellar biosynthesis sigma factor
1958	SL1887	*fliB*	-6,30407	-6,61295	lysine-N-methylase

1959	SL1888	*fliC*	-6,07778	-6,55312	flagellin
1960	SL1889	*fliD*	-8,24866	-9,03107	flagellar capping protein
1961	SL1890	*fliS*	-6,72678	-7,00214	flagellar protein FliS
1962	SL1891	*fliT*	-6,45004	-6,44869	flagellar biosynthesis protein FliT
1968	SL1897	*fliE*	-4,40061	-3,87333	flagellar hook-basal body protein FliE
1969	SL1898	*fliF*	-4,52256	-4,49656	flagellar MS-ring protein
1970	SL1899	*fliG*	-5,80939	-5,364	flagellar motor switch protein G
1971	SL1900	*fliH*	-5,15984	-4,3348	flagellar assembly protein H
1972	SL1901	*fliI*	-4,60108	-3,78864	flagellum-specific ATP synthase
1973	SL1902	*fliJ*	-7,35072	-7,3361	flagellar biosynthesis chaperone
1974	SL1903	*fliK*	-4,67108	-4,68336	flagellar hook-length control protein
1975	SL1904	*fliL*	-3,70536	-2,52217	flagellar basal body-associated protein FliL
1976	SL1905	*fliM*	-5,91778	-5,91502	flagellar motor switch protein FliM
1977	SL1906	*fliN*	-6,10392	-5,42285	flagellar motor switch protein FliN
1978	SL1907	*fliO*	-4,86694	-4,27314	flagellar biosynthesis protein FliO
1979	SL1908	*fliP*	-2,35408	-1,64851	flagellar biosynthesis protein FliP
2771	SL2756	*fljB*	-6,99806	-7,45804	flagellin
			CHEMOTAXIS		
1917	SL1852	*cheB*	-6,79446	-6,055	chemotaxis-specific methylesterase
1918	SL1853	*cheR*	-6,644	-6,09016	chemotaxis methyltransferase CheR
1919	SL1854	*cheM*	-8,83596	-8,75253	methyl accepting chemotaxis protein II
1920	SL1855	*cheW*	-7,31736	-6,24275	purine-binding chemotaxis protein
1921	SL1856	*cheA*	-7,97546	-7,29865	chemotaxis protein CheA
3217	SL3190	*aer*	-6,54713	-6,34625	aerotaxis sensor receptor
1626	SL1556	*trg*	-7,06638	-7,19467	methyl-accepting chemotaxis protein III
			METABOLISM		
781	SL0758	*modA*	-2,42126	-2,48426	molybdate transporter periplasmic protein
782	SL0759	*modB*	-2,4873	-2,4489	molybdate ABC transporter permease protein
783	SL0760	*modC*	-2,68069	-2,87297	molybdate transporter ATP-binding protein
800	SL0776	*slrp*	-2,19948	-2,52212	leucine-rich repeat-containing protein
2548	SL2510	*asrA*	-2,44988	-2,84987	anaerobic sulfide reductase
2549	SL2511	*asrB*	-2,95773	-1,98773	anaerobic sulfite reductase subunit B
2550	SL2512	*asrC*	-2,30442	-1,36859	anaerobic sulfide reductase
2558	SL2520	*cadB*	-3,2369	-2,8327	lysine/cadaverine antiporter
2559	SL2521	*cadA*	-2,92782	-2,81164	lysine decarboxylase 1
	SL2674	*sopE*	-9,40074	-9,35711	
2773	SL2758	*iroB*	-3,84752	-3,49264	putative glycosyl transferase
2774	SL2759	*iroC*	-3,47552	-2,68924	putative ABC transporter protein
2775	SL2760	*iroD*	-2,19228	-1,41579	enterochelin esterase=-like protein
2776	SL2761	*iroE*	-2,35303	-1,39884	putative hydrolase
2878	SL2858	*sptP*	-2,89798	-3,0085	protein tyrosine phosphatase/GTPase activating protein

3189	SL3163	*ygiD*	-2,39064	-2,08937	hypothetical protein-seems to be involved in biofilm formation
3240	SL3212	*tdcG*	-2,02863	-1,36615	L-serine deaminase
3241	SL3213	*tdcE*	-2,67232	-1,6187	pyruvate formate-lyase 4/2-ketobutyrate formate-lyase
3577	SL3542	*tcp*	-8,57565	-8,41645	methyl-accepting transmembrane citrate/phenol chemoreceptor
3578	SL3543	*yhhP*	-3,28923	-3,91009	cell developmental protein SirA
3611	SL3576	*yhjH*	-6,16238	-6,69775	EAL domain-containing protein
			OTHERS		
1301	SL1236		-2,67513	-2,89511	
1328	SL1263		-6,18503	-5,68145	
1330	SL1265		-2,2161	-1,42279	
	SL1501		-2,01195	-1,10347	
1596	SL1527	*ydcX*	-2,25657	-1,78552	putative inner membrane protein
1629	SL1559	*steB*	-3,45928	-2,85784	
1798	SL1726	*ycgR*	-6,24285	-6,25779	putative inner membrane protein
1934	SL1867		-2,27948	-2,08313	
1915	SL1879	*sliA*	-2,62839	-2,6717	
1967	SL1896		-2,22395	-1,96412	
2314	SL2283		-7,64248	-7,17453	
2879	SL2859	*sipP*	-3,56567	-3,63951	
2881	SL2860	*iacP*	-6,58496	-6,92133	
3112	SL3112		-6,15379	-6,18479	
3151	SL3125	*yghW*	-2,84758	-2,29981	putative cytoplasmic protein
3152	SL3126		-5,78976	-5,54493	
3154	SL3128		-3,41389	-2,63399	
3155	SL3129		-2,94511	-3,59618	
3156	SL3130		-3,87149	-4,01726	
3216	SL3189		-8,24609	-8,31326	
3604	SL3569		-3,16878	-2,62134	
4263	SL4199	*yjcB*	-2,02875	-1,72748	putative inner membrane protein
4310	SL4247		-2,93994	-2,68906	
4312	SL4248		-3,24214	-3,42733	
4313	SL4249		-4,27317	-4,01976	
4314	SL4250		-4,38762	-3,91761	rtsB
	SL4251		-5,67293	-5,46996	
	SL4464		-6,92337	-7,12987	

Table SM. 2 ChIP-seq results

S. enterica	Peak start	Peak end	Map position		Gene description	Strand
StdE binding peaks						
SL1344	133850	133999	133829	133915	WP_001575587.1_leu_operon_leader_peptide	-
SL1344	139700	140049	139950	140408	WP_000488294.1_transcriptional_regulator_MraZ	+
SL1344	174750	175049	173384	174754	WP_000378215.1_aromatic_amino_acid_transporter	-
SL1344	216850	217049	217026	217730	WP_000899412.1_sugar_fermentation_stimulation_protein_A	-
SL1344	223500	223799	223735	225924	WP_000113220.1_ferrichrome_outer_membrane_transporter	+
SL1344	242800	243049	242841	244358	WP_000146443.1_deoxyguanosinetriphosphate_triphosphohydrolase	+
SL1344	247700	247849	247314	247700	WP_000272193.1_hypothetical_protein	-
SL1344	250050	250249	250139	252811	WP_001094519.1_uridylyltransferase	-
SL1344	259000	259099	257842	259038	WP_000811905.1_1-deoxy-D-xylulose_5-phosphate_reductoisomerase	+
SL1344	265850	265949	265401	266426	WP_001139265.1_UDP-3-O-(3-hydroxymyristoyl)glucosamine_N-acyltransferase_lpxD	+
SL1344	288800	289099	288235	288801	WP_001051726.1_D-glycero-beta-D-manno-heptose-1,7-bisphosphate_7-phosphatase	+
SL1344	304200	304299	304258	304334	tRNA-Asp_tRNA-Asp	+
SL1344	368100	368299	368274	368349	tRNA-Thr_tRNA-Thr	+
SL1344	386200	386549	385794	386294	WP_001539242.1_hypothetical_protein	+
SL1344	403400	403699	402817	404775	WP_000910371.1_type_III_restriction-modification_system_StyLTI_enzyme_mod	+
SL1344	413700	413849	412167	414257	WP_001526309.1_ferrioxamine_B_receptor	+
SL1344	414850	415049	414298	414930	WP_000433131.1_transporter	-
SL1344	434800	435099	435083	436294	WP_000446768.1_permease	+
SL1344	454400	454499	454138	455508	WP_001555879.1_proline-specific_permease_ProY_proY	+
SL1344	461150	461549	461457	461789	WP_000007628.1_preprotein_translocase_subunit_YajC_yajC	+
SL1344	498400	498499	498086	499561	WP_000098481.1_AmpG_family_muropeptide_MFS_transporter_ampG	-
SL1344	507800	508149	507591	507863	WP_001043544.1_DNA-binding_protein_HU-beta	+
SL1344	527350	527649	527619	527837	WP_001280991.1_hemolysin_expression-modulating_protein_Hha	-

SL1344	531750	532149	531907	533100	WP_001039202.1_MexE_family_multidrug_efflux_RND_transporter_periplasmic_adaptor_subunit	-
SL1344	555350	555649	555572	556366	WP_000421859.1_hypothetical_protein	-
SL1344	643500	643649	643490	644704	WP_000656600.1_enterobactin/ferric_enterobactin_esterase	+
SL1344	660450	660699	660167	660580	WP_000637965.1_proofreading_thioesterase_EntH	+
SL1344	677300	677499	676860	677288	WP_000278499.1_universal_stress_protein_G	-
SL1344	677300	677499	677511	678749	WP_000646113.1_dehydrogenase	+
SL1344	692200	692549	692403	692612	WP_000034826.1_cold-shock_protein	+
SL1344	701350	701699	701433	703334	WP_000828700.1_penicillin-binding_protein_2	-
SL1344	725850	726049	726021	726746	WP_000631369.1_glutamate/aspartate_ABC_transporter_ATP-binding_protein_artP	-
SL1344	738000	738349	738051	738127	tRNA-Met_tRNA-Met	-
SL1344	749000	749149	748945	750351	WP_001258803.1_chitoporin_chiP	+
SL1344	749800	749999	748945	750351	WP_001258803.1_chitoporin_chiP	+
SL1344	765400	765699	765386	765499	WP_000862019.1_hypothetical_protein	+
SL1344	768550	769049	766424	769108	WP_000997487.1_two-component_system_sensor_histidine_kinase_KdpD	-
SL1344	797050	797199	796608	796994	WP_010988982.1_membrane_protein	+
SL1344	808350	808449	808940	810508	WP_000884369.1_cytochrome_BD_oxidase_subunit_I	+
SL1344	822850	823049	823028	824080	WP_001109234.1_phospho-2-dehydro-3-deoxyheptonate_aldolase	+
SL1344	827300	827549	826600	827526	WP_001538325.1_LysR_family_transcriptional_regulator	-
SL1344	847500	847749	847453	848271	WP_000127031.1_pyridoxal_phosphate_phosphatase	-
SL1344	865300	865499	863688	865709	WP_000042502.1_UvrABC_system_protein_B	+
SL1344	869650	870049	869893	870882	WP_000168180.1_cyclic_pyranopterin_monophosphate_synthase_moaA	+
SL1344	878400	878599	878496	878906	WP_000871970.1_membrane_protein	+
SL1344	904200	904299	904297	905889	WP_000961478.1_heme_ABC_transporter_ATP-binding_protein	+
SL1344	913600	913999	913046	913945	WP_000576947.1_pyruvate_formate_lyase_activating_enzyme	-
SL1344	964050	964299	964160	965593	WP_000069000.1_hypothetical_protein	-
SL1344	965450	965599	964160	965593	WP_000069000.1_hypothetical_protein	-
SL1344	974900	975099	974052	975002	WP_001519667.1_virulence_protein_VirK	-

SL1344	978650	978849	978884	979204	WP_000520789.1_ATP-dependent_Clp_protease_adapter_protein_ClpS_clpS	+
SL1344	1017750	1018049	1018042	1019130	WP_000079584.1_phosphoserine_aminotransferase	+
SL1344	1022000	1022349	1021561	1022244	WP_000125006.1_cytidylate_kinase_cmk	+
SL1344	1043350	1043449	1041587	1043428	WP_000925872.1_murein_transpeptidase	+
SL1344	1043850	1044099	1043688	1044236	WP_000357052.1_outer_membrane_protein	+
SL1344	1050000	1050299	1050105	1050338	WP_000301921.1_hypothetical_protein	-
SL1344	1068800	1069049	1068940	1069257	WP_010989004.1_hypothetical_protein	+
SL1344	1086800	1087199	1086605	1087138	WP_000877926.1_Superoxide_dismutase_[Cu-Zn]_1	-
SL1344	1089650	1090049	1089344	1092694	WP_000033415.1_host_specificity_protein_J	+
SL1344	1110650	1110749	1110249	1111502	WP_000333148.1_paraquat-inducible_protein_A	+
SL1344	1113800	1113899	1113963	1114130	WP_001537784.1_ribosome_modulation_factor	+
SL1344	1115900	1116199	1114817	1116577	WP_000156453.1_lon_protease	-
SL1344	1131500	1131799	1131644	1131731	tRNA-Ser_tRNA-Ser	+
SL1344	1158750	1159049	1158770	1159393	WP_000876693.1_DSBA_oxidoreductase	+
SL1344	1167950	1168199	1163919	1167881	WP_000537528.1_bifunctional_protein_PutA_putA	-
SL1344	1183450	1183699	1183644	1184126	WP_001546652.1_membrane_protein	+
SL1344	1192350	1192599	1192541	1194094	WP_000681121.1_glucan_biosynthesis_protein_D_mdoG	+
SL1344	1262600	1262749	1261987	1262898	WP_000291338.1_N-acetyl-D-glucosamine_kinase	+
SL1344	1289850	1290099	1290067	1290143	tRNA-Arg_tRNA-Arg	+
SL1344	1306350	1306599	1306474	1306623	WP_000908464.1_hypothetical_protein	-
SL1344	1309700	1310099	1309991	1310239	WP_000512149.1_membrane_protein	+
SL1344	1326600	1326849	1326883	1327296	WP_001519539.1_peptide_methionine_sulfoxide_reductase_MsrB	+
SL1344	1383250	1383449	1382251	1383297	WP_001082196.1_phospho-2-dehydro-3-deoxyheptonate_aldolase_Trp-sensitive	-
SL1344	1399850	1400149	1399804	1401015	WP_000797677.1_transporter	-
SL1344	1482900	1483049	1482592	1483197	WP_000765727.1_glutathionine_S-transferase	-
SL1344	1491950	1492249	1491858	1492298	WP_000214061.1_membrane_protein	-
SL1344	1510850	1511099	1511078	1512022	WP_000769294.1_hypothetical_protein	-

SL1344	1544700	1544849	1544840	1545526	WP_000215563.1_hypothetical_protein	-
SL1344	1550600	1550949	1550924	1551019	WP_000901531.1_membrane_protein	-
SL1344	1556950	1557099	1557061	1557933	WP_000366538.1_LysR_family_transcriptional_regulator	-
SL1344	1589900	1590099	1588983	1589990	WP_000201080.1_transcriptional_regulator	+
SL1344	1611850	1612099	1612026	1613114	WP_000769035.1_porin	+
SL1344	1615100	1615599	1615267	1615845	WP_000121043.1_TetR_family_transcriptional_regulator	+
SL1344	1637850	1638049	1637948	1640929	WP_000960833.1_virulence_factor_SrfB	+
SL1344	1643250	1643599	1643115	1643288	WP_000171943.1_hypothetical_protein	-
SL1344	1658450	1658649	1658651	1659769	WP_000144617.1_aminopeptidase	+
SL1344	1706800	1706949	1707001	1707984	WP_000387373.1_zinc_transporter_ZntB_zntB	-
SL1344	1725400	1725549	1724854	1725567	WP_000171963.1_oxidoreductase	+
SL1344	1736100	1736199	1735781	1737178	WP_000825847.1_ATPase	-
SL1344	1743250	1743549	1743130	1744020	WP_001146150.1_peptide_ABC_transporter_permease	+
SL1344	1749900	1750149	1749977	1751110	WP_000434197.1_hypothetical_protein	+
SL1344	1759850	1759999	1759544	1759852	WP_000876301.1_membrane_protein	-
SL1344	1764650	1764949	1764839	1764967	WP_001138871.1_hypothetical_protein	-
SL1344	1802000	1802349	1799981	1802659	WP_000301678.1_acetaldehyde_dehydrogenase	+
SL1344	1803850	1804049	1804136	1804549	WP_001287383.1_DNA-binding_protein_H-NS	+
SL1344	1834000	1834299	1833497	1834348	WP_000988246.1_4-diphosphocytidyl-2-C-methyl-D-erythritol_kinase	+
SL1344	1837200	1837349	1835686	1837371	WP_001037181.1_transporter	+
SL1344	1837600	1837799	1837416	1837694	WP_000823878.1_membrane_protein	-
SL1344	1863400	1863649	1862742	1863461	WP_000234826.1_fatty_acid_metabolism_regulator_protein	-
SL1344	1893350	1893799	1893337	1893480	WP_001537930.1_hypothetical_protein	-
SL1344	1894500	1894749	1894487	1894630	WP_000714547.1_PhoP_family_transcriptional_regulator	-
SL1344	1901600	1901899	1901554	1902051	WP_001518229.1_GAF_domain_protein	-
SL1344	1930600	1930799	1929373	1931424	WP_000936999.1_protease	-
SL1344	1935150	1935399	1935189	1937000	WP_001069113.1_phosphogluconate_dehydratase	-

SL1344	1979550	1980099	1979284	1979625	WP_001518146.1_flagellar_transcriptional_regulator_FlhD	-
SL1344	1980200	1980549	1980421	1980849	WP_000122606.1_universal_stress_protein_C	+
SL1344	1983000	1983399	1982263	1983066	WP_000830110.1_trehalose-phosphate_phosphatase	-
SL1344	2034450	2034599	2034188	2034454	WP_001521850.1_membrane_protein	-
SL1344	2039600	2039849	2039277	2039438	WP_000500831.1_hypothetical_protein	-
SL1344	2059350	2059549	2058604	2060337	WP_000088182.1_terminase	-
SL1344	2064750	2064899	2064878	2065501	WP_000188927.1_hypothetical_protein	-
SL1344	2133550	2133799	2133740	2134912	WP_000925042.1_D-alanyl-D-alanine_carboxypeptidase	-
SL1344	2134850	2135149	2135036	2135800	WP_001092547.1_thiosulfate_reductase_cytochrome_B	-
SL1344	2139050	2139249	2139285	2141633	WP_000703998.1_E3_ubiquitin--protein_ligase	+
SL1344	2147100	2147249	2147330	2148229	WP_000886607.1_ATP_phosphoribosyltransferase_hisG	+
SL1344	2153950	2154199	2153855	2154466	WP_000954848.1_histidine_biosynthesis_bifunctional_protein_HisIE	+
SL1344	2155300	2155399	2154547	2155530	WP_000215261.1_chain_length_determinant_protein	-
SL1344	2177050	2177299	2176672	2177565	WP_000981469.1_UTP--glucose-1-phosphate_uridylyltransferase	-
SL1344	2198800	2198949	2198772	2199221	WP_000482224.1_protein-tyrosine_phosphatase	-
SL1344	2211500	2211749	2210207	2211559	WP_000469629.1_chaperone	+
SL1344	2234750	2234899	2233987	2235258	WP_000858692.1_nucleoside_permease	+
SL1344	2255700	2255849	2255678	2256625	WP_000569166.1_ABC_transporter_ATP-binding_protein	-
SL1344	2264450	2264699	2264354	2264941	WP_001017057.1_transporter	-
SL1344	2266300	2266599	2266545	2267981	WP_001081453.1_lipoprotein	-
SL1344	2276650	2276949	2276882	2277766	WP_000553526.1_cytidine_deaminase	+
SL1344	2286900	2287149	2287003	2288145	WP_001526153.1_membrane_protein	-
SL1344	2289600	2289699	2289158	2290015	WP_000425488.1_S-formylglutathione_hydrolase	+
SL1344	2305050	2305299	2303940	2305070	WP_000487287.1_multiphosphoryl_transfer_protein	-
SL1344	2311150	2311399	2311374	2311946	WP_000241015.1_membrane_protein_spr	+
SL1344	2317850	2318149	2317871	2319460	WP_000203622.1_microcin_C_ABC_transporter_ATP-binding_protein_YejF	+
SL1344	2332500	2332899	2332550	2333077	WP_001215679.1_tail_protein	-

SL1344	2357000	2357449	2356870	2357361	WP_000228070.1_ferredoxin	-
SL1344	2373950	2374199	2371427	2374063	WP_001281271.1_DNA_gyrase_subunit_A	-
SL1344	2384150	2384299	2384252	2385130	WP_000176719.1_LysR_family_transcriptional_regulator	+
SL1344	2387600	2388149	2387952	2389580	WP_000857292.1_sn-glycerol-3-phosphate_dehydrogenase_subunit_A_glpA	+
SL1344	2406000	2406249	2405788	2406687	WP_000169761.1_4-deoxy-4-formamido-L-arabinose-phosphoundecaprenol_deformylase_ArnD	+
SL1344	2437500	2437749	2437609	2437761	WP_001522276.1_hypothetical_protein	-
SL1344	2445400	2445499	2444860	2445315	WP_000106617.1_membrane_protein	-
SL1344	2450350	2450599	2449296	2450816	WP_000117932.1_hypothetical_protein	+
SL1344	2475150	2475449	2475070	2476338	WP_000792275.1_bifunctional_folylpolyglutamate_synthase/_dihydrofolate_synthase	-
SL1344	2499800	2499949	2499456	2499740	WP_000030904.1_hypothetical_protein	-
SL1344	2508800	2509249	2508217	2509491	WP_031602386.1_ABC_transporter_substrate-binding_protein	-
SL1344	2509400	2509649	2509454	2509672	WP_000279836.1_hypothetical_protein	-
SL1344	2522750	2523149	2523019	2524221	WP_000376347.1_nucleoside_permease	+
SL1344	2526750	2526999	2526772	2526847	tRNA-Ala_tRNA-Ala	-
SL1344	2539750	2539899	2538804	2539790	WP_000983110.1_cell_division_protein_ZipA	-
SL1344	2541850	2542099	2542037	2542162	WP_000718558.1_hypothetical_protein	-
SL1344	2582450	2582599	2581216	2583216	WP_000087323.1_transketolase	+
SL1344	2595600	2595999	2595410	2596537	WP_001277825.1_succinyl-diaminopimelate_desuccinylase	+
SL1344	2602550	2602799	2602783	2603355	WP_000189061.1_glycine_cleavage_system_transcriptional_regulator_gcvR	+
SL1344	2610200	2610399	2609749	2610375	WP_000706208.1_uracil_phosphoribosyltransferase_upp	-
SL1344	2618650	2618949	2618927	2619118	WP_000075924.1_hypothetical_protein	+
SL1344	2623600	2623899	2623795	2625144	WP_000953165.1_exodeoxyribonuclease_7_large_subunit_xseA	+
SL1344	2647300	2647549	2646325	2648517	WP_000252220.1_intimin	-
SL1344	2661250	2661649	2660629	2663007	WP_001521637.1_DMSO_reductase_subunit_A	-
SL1344	2681950	2682149	2681302	2682033	WP_000940032.1_tRNA_(cytidine/uridine-2'-O-)-methyltransferase_TrmJ	-
SL1344	2705350	2705599	2703891	2705276	WP_001542313.1_two-component_system_sensor_histidine_kinase	-
SL1344	2706400	2706499	2705971	2709858	WP_000970045.1_phosphoribosylformylglycinamidine_synthase	-

SL1344	2718850	2719049	2718443	2719369	WP_001022463.1_LysR_family_transcriptional_regulator	+
SL1344	2759600	2759849	2759753	2760316	WP_000211410.1_antirepressor	-
SL1344	2760450	2760599	2760589	2761266	WP_001097241.1_antitermination_protein	-
SL1344	2762700	2762799	2762220	2762822	WP_000929790.1_hypothetical_protein	-
SL1344	2768350	2768599	2768366	2768833	WP_000169863.1_regulator	+
SL1344	2790150	2790349	2790293	2792953	WP_000082639.1_protein_lysine_acetyltransferase_Pat	+
SL1344	2792900	2793049	2790293	2792953	WP_000082639.1_protein_lysine_acetyltransferase_Pat	+
SL1344	2802200	2802649	2802529	2805102	WP_001235094.1_chaperone_protein_ClpB	-
SL1344	2807750	2808149	2808081	2808419	WP_000178449.1_translation_inhibitor_protein_RaiA	+
SL1344	2821400	2821649	2820345	2822183	partial;pseudo_partial;pseudo	+
SL1344	2838150	2838249	2837672	2838154	WP_001518569.1_SsrA-binding_protein_smpB	+
SL1344	2855100	2855499	2853874	2855064	WP_000700669.1_secretion_protein_HlyD	+
SL1344	2887950	2888199	2887497	2888522	WP_000155500.1_recombinase	+
SL1344	2935050	2935349	2935199	2935738	WP_000388997.1_repressor_of_phase_1_flagellin_gene	-
SL1344	2956900	2957049	2956337	2957011	WP_001237934.1_two-component_system_response_regulator	-
SL1344	2973900	2974099	2973730	2974161	WP_000209813.1_alkylhydroperoxidase	+
SL1344	3041050	3041249	3040412	3041341	WP_000432699.1_transcriptional_regulator_hilD	+
SL1344	3050450	3050599	3050433	3051662	WP_000909019.1_cell_invasion_protein_SipC	-
SL1344	3084250	3084549	3084385	3085149	WP_000613185.1_DeoR_family_transcriptional_regulator	+
SL1344	3089450	3090199	3089110	3090243	WP_001272632.1_murein_hydrolase_activator_NlpD_nlpD	-
SL1344	3137150	3137399	3137007	3137789	WP_000890027.1_tRNA_pseudouridine_synthase_C	-
SL1344	3138100	3138599	3138274	3138486	WP_000988723.1_hypothetical_protein	-
SL1344	3139050	3139149	3138730	3139275	WP_000343990.1_protein_Syd	-
SL1344	3156400	3156949	3155734	3156651	WP_000044409.1_transcriptional_regulator	-
SL1344	3162300	3162549	3161221	3162318	WP_000678626.1_murein_transglycosylase_mltA	-
SL1344	3166600	3166799	3165738	3167573	WP_000155129.1_exonuclease_V_subunit_alpha_recD	-
SL1344	3181050	3181199	3180280	3181155	WP_000204645.1_prolipoprotein_diacylglyceryl_transferase	-

SL1344	3184300	3184549	3184511	3184657	WP_001752593.1_hypothetical_protein	-
SL1344	3205500	3205949	3205639	3206475	WP_000503692.1_cobalt_transporter yohM	+
SL1344	3215550	3215899	3215876	3216274	WP_000911336.1_tRNA(fMet)-specific_endonuclease_VapC	-
SL1344	3242700	3243149	3243022	3243618	WP_001520956.1_5-formyltetrahydrofolate_cyclo-ligase	+
SL1344	3243550	3243799	3243022	3243618	WP_001520956.1_5-formyltetrahydrofolate_cyclo-ligase	+
SL1344	3247250	3247349	3247215	3247466	WP_001112716.1_hypothetical_protein	+
SL1344	3253650	3253949	3252597	3253643	WP_000218338.1_D-erythrose-4-phosphate_dehydrogenase_gapA	-
SL1344	3259700	3260099	3259380	3260138	WP_000701830.1_metalloprotease	+
SL1344	3267550	3267699	3267369	3267485	WP_001738517.1_racemase	+
SL1344	3291950	3292099	3290856	3291941	WP_000976289.1_murein_transglycosylase_mltC	+
SL1344	3330950	3331099	3329490	3331193	WP_000083044.1_hydrogenase_2_large_subunit	-
SL1344	3341350	3341549	3341439	3341576	WP_001518378.1_membrane_protein	-
SL1344	3350700	3350999	3349642	3351813	WP_001274458.1_radical_SAM_protein	-
SL1344	3383000	3383299	3383264	3384697	WP_000867682.1_bifunctional_protein_HldE	-
SL1344	3385050	3385499	3384745	3387588	WP_000188309.1_glutamate--ammonia-ligase_adenylyltransferase	-
SL1344	3394450	3394849	3394760	3394975	WP_001144069.1_30S_ribosomal_protein_S21_rpsU	+
SL1344	3399500	3399849	3399709	3399784	tRNA-Met_tRNA-Met	+
SL1344	3409000	3409249	3409059	3410195	WP_000019989.1_ribosomal_RNA_large_subunit_methyltransferase_G	-
SL1344	3424950	3425049	3424056	3425387	WP_000443187.1_membrane_protein	-
SL1344	3434600	3434749	3433178	3434116	WP_001094910.1_transcriptional_regulator	-
SL1344	3435800	3435999	3435915	3437060	WP_000706459.1_glycerate_kinase	-
SL1344	3443000	3443149	3442143	3442976	WP_001118363.1_DeoR_family_transcriptional_regulator	-
SL1344	3447250	3447649	3447534	3448805	WP_000658628.1_tagatose-1,6-bisphosphate_aldolase	+
SL1344	3452000	3452249	3452174	3452947	WP_001083896.1_galactitol_utilization_operon_repressor	+
SL1344	3468800	3469049	3468958	3469842	WP_000802069.1_lipoprotein_NlpI	-
SL1344	3469200	3469749	3468958	3469842	WP_000802069.1_lipoprotein_NlpI	-
SL1344	3479100	3479549	3479208	3479284	tRNA-Met_tRNA-Met	-

SL1344	3511700	3511949	3511909	3514245	WP_000809815.1_aerobic_respiration_control_sensor_protein_ArcB	-
SL1344	3515550	3515699	3515802	3516017	WP_001546060.1_hypothetical_protein	-
SL1344	3522550	3522749	3522035	3523138	WP_000184315.1_hypothetical_protein	+
SL1344	3530400	3530699	3530564	3531355	WP_000382926.1_transcriptional_regulator_NanR	-
SL1344	3532700	3532849	3532871	3533665	WP_000505557.1_hypothetical_protein	-
SL1344	3550150	3550299	3550283	3550546	WP_000695693.1_outer_membrane_protein	+
SL1344	3578500	3578649	3578064	3578948	WP_000642611.1_methyltransferase	+
SL1344	3593550	3593749	3593756	3593947	WP_016696011.1_hypothetical_protein	-
SL1344	3597750	3598299	3597162	3598286	WP_000124529.1_hypothetical_protein	-
SL1344	3618000	3618349	3617701	3618012	WP_001181005.1_30S_ribosomal_protein_S10_rpsJ	-
SL1344	3636800	3637099	3636416	3636820	WP_001148919.1_hypothetical_protein	-
SL1344	3643350	3643549	3643554	3644735	WP_000185211.1_protein_TsgA	+
SL1344	3664250	3664549	3663746	3664267	WP_000818621.1_shikimate_kinase_aroK	-
SL1344	3671300	3671549	3670820	3671383	WP_000045725.1_adenosine_nucleotide_hydrolase_nudE	-
SL1344	3677700	3678099	3678085	3679704	WP_001265689.1_phosphoenolpyruvate_carboxykinase_[ATP]	+
SL1344	3685200	3685499	3685412	3685639	WP_001160955.1_ferrous_iron_transporter_A_feoA	+
SL1344	3698350	3698449	3698422	3701127	WP_000907029.1_transcriptional_regulator	+
SL1344	3714950	3715149	3713815	3714924	WP_000258838.1_glycerol_dehydrogenase_gldA	-
SL1344	3736700	3736949	3736939	3737427	WP_001290288.1_acetyltransferase	+
SL1344	3749800	3749949	3749623	3749844	WP_001520924.1_prevent-host-death_family_protein	-
SL1344	3758050	3758399	3758349	3759404	WP_001081707.1_cell_division_protein_FtsX_ftsX	-
SL1344	3796650	3796899	3796899	3798548	WP_000934257.1_trehalase_treF	+
SL1344	3804050	3804349	3802998	3804026	WP_000191231.1_membrane_protein	+
SL1344	3829450	3829799	3829506	3829613	WP_001669334.1_hypothetical_protein	-
SL1344	3837250	3837499	3835747	3837354	WP_000028047.1_peptide_ABC_transporter_substrate-binding_protein	-
SL1344	3841750	3841949	3841901	3843592	WP_001269259.1_phosphoethanolamine_transferase_EptB	-
SL1344	3857550	3857749	3857754	3857966	WP_000014594.1_cold-shock_protein	+

SL1344	3871550	3871749	3871475	3871846	WP_000254414.1_hypothetical_protein	+
SL1344	3896100	3896599	3896582	3898498	WP_000093287.1_PTS_mannitol_transporter_subunit_IIABC	+
SL1344	3902950	3903399	3902543	3906928	WP_001079584.1_membrane_protein	+
SL1344	3912600	3912799	3912770	3913966	WP_001218249.1_L-talarate/galactarate_dehydratase	+
SL1344	3921900	3922099	3921719	3922681	WP_001135518.1_hypothetical_protein	+
SL1344	3945900	3946299	3946033	3946491	WP_000976078.1_deoxyuridine_5'-triphosphate_nucleotidohydrolase	+
SL1344	3951700	3951949	3951856	3952473	WP_000924334.1_membrane_protein	+
SL1344	3957300	3957549	3957500	3958189	WP_001068438.1_tRNA_methyltransferase	+
SL1344	3999850	4000099	3998699	3999891	pseudo;old_locus_tag=SL1344_3741_pseudo;old_locus_tag=SL1344_3741_nepI	-
SL1344	4006550	4006699	4005752	4006495	WP_000854956.1_GntR_family_transcriptional_regulator	-
SL1344	4019000	4019399	4019024	4019122	WP_001541152.1_ilvB_operon_leader_peptide	-
SL1344	4045350	4045949	4044666	4045850	WP_001230254.1_trimethylamine_N-oxide_reductase_cytochrome_C_subunit	-
SL1344	4058750	4058949	4057949	4059139	WP_000705615.1_MR-MLE_family_protein	+
SL1344	4066700	4066999	4066999	4067139	WP_000831330.1_50S_ribosomal_protein_L34_rpmH	+
SL1344	4072450	4072549	4071657	4072364	WP_001738600.1_recombinase	+
SL1344	4101950	4102249	4101487	4101867	WP_000116685.1_F0F1_ATP_synthase_subunit_I	-
SL1344	4126700	4126949	4126913	4126988	tRNA-Trp_tRNA-Trp	+
SL1344	4127900	4128049	4128045	4128383	WP_000840996.1_hypothetical_protein hdfR	+
SL1344	4130150	4130449	4130280	4130378	WP_001311244.1_IlvGMEDA_operon_leader_peptide	+
SL1344	4146700	4147149	4147129	4148388	WP_001054532.1_transcription_termination_factor_Rho_rho	+
SL1344	4167700	4167899	4167912	4170458	WP_000281718.1_adenylate_cyclase_cyaA	+
SL1344	4196200	4196449	4196409	4197170	WP_000045169.1_uridine_phosphorylase	+
SL1344	4227150	4227549	4226273	4227181	WP_000196890.1_acyltransferase	-
SL1344	4230250	4230499	4227569	4230355	WP_000249972.1_DNA_polymerase_I	+
SL1344	4231650	4231799	4231562	4231786	WP_001541209.1_hypothetical_protein	+
SL1344	4238550	4238899	4238822	4240645	WP_000572067.1_GTP-binding_protein	+
SL1344	4244000	4244249	4243537	4244412	WP_000282794.1_membrane_protein	-

SL1344	4252950	4253299	4253044	4254285	WP_000870946.1_sulfoquinovose_isomerase	-
SL1344	4280300	4280449	4280503	4281339	WP_000217112.1_transcriptional_activator_RhaS	+
SL1344	4283500	4283849	4283840	4283965	WP_000183584.1_hypothetical_protein	+
SL1344	4284700	4284999	4284831	4286138	WP_000566800.1_membrane_protein	-
SL1344	4321150	4321249	4320588	4321919	WP_001293360.1_ATP-dependent_protease_ATPase_subunit_HslU_hslU	-
SL1344	4348350	4348749	4348739	4349818	WP_000661597.1_PTS_fructose_transporter_subunit_IIC	+
SL1344	4381250	4381449	4381377	4381452	tRNA-Thr_tRNA-Thr	+
SL1344	4384150	4384499	4384436	4384864	WP_001085926.1_50S_ribosomal_protein_L11	+
SL1344	4385450	4385699	4384868	4385572	WP_001096676.1_50S_ribosomal_protein_L1	+
SL1344	4407800	4408099	4407263	4407853	WP_000940092.1_hypothetical_protein	+
SL1344	4415600	4415949	4416006	4417559	16S_ribosomal_RNA_16S_ribosomal_RNA	+
SL1344	4471800	4472149	4470620	4471810	WP_000695417.1_maltose-binding_periplasmic_protein_malE	-
SL1344	4479250	4479499	4477472	4479892	WP_000017359.1_glycerol-3-phosphate_acyltransferase	-
SL1344	4497400	4497549	4497295	4497411	WP_001600633.1_hypothetical_protein	+
SL1344	4523150	4523349	4522829	4523110	WP_000719058.1_membrane_protein	-
SL1344	4524150	4524299	4523673	4525274	WP_000083640.1_hypothetical_protein	+
SL1344	4537050	4537149	4537151	4537297	WP_001576543.1_entericidin_B_precursor	+
SL1344	4544800	4545049	4544308	4545618	WP_000793278.1_proton_glutamate_symport_protein	+
SL1344	4569700	4569899	4569886	4570152	WP_010989095.1_hypothetical_protein	-
SL1344	4572450	4572599	4571185	4572816	WP_000682911.1_sensor_histidine_kinase	-
SL1344	4587200	4587299	4587160	4587235	tRNA-Phe_tRNA-Phe	-
SL1344	4593000	4593299	4592995	4593117	WP_000614819.1_hypothetical_protein	-
SL1344	4594850	4595199	4593838	4595079	WP_001670701.1_transporter	-
SL1344	4639450	4639599	4638946	4640109	WP_000943932.1_glutathionylspermidine_synthase	+
SL1344	4642300	4642699	4642267	4642596	WP_000586836.1_transporter	-
SL1344	4650900	4651049	4650793	4651023	WP_001621821.1_hypothetical_protein	+
SL1344	4651600	4651699	4651496	4651810	WP_001519453.1_primosomal_replication_protein_n	+

SL1344	4655150	4655299	4654512	4655132	WP_000235161.1_peptidyl-prolyl_cis-trans_isomerase	+
SL1344	4704200	4704499	4703860	4704246	WP_001232231.1_soluble_cytochrome_b562	+
SL1344	4719850	4720149	4718940	4719887	WP_001181297.1_transcriptional_regulator_treR	-
SL1344	4749550	4749649	4748546	4749577	WP_000453340.1_L-idonate_5-dehydrogenase	-
SL1344	4776350	4776599	4776160	4776417	WP_001054380.1_hypothetical_protein	-
SL1344	4810450	4810599	4810665	4812326	WP_000919519.1_methyl-accepting_chemotaxis_protein	+
SL1344	4836200	4836449	4836462	4837079	WP_000178963.1_hypothetical_protein	+
SL1344	4838900	4839199	4838563	4839336	WP_000119016.1_deoxyribonuclease	+
SL1344	4841750	4841999	4840204	4841751	WP_001111692.1_hypothetical_protein	-
pSLT	29800	30149	29946	30512	WP_000399768.1_conjugal_transfer_protein_TraE	-
pSLT	46500	46649	46488	47762	WP_000457541.1_DNA_polymerase_V_subunit_UmuC	+
pSLT	47150	47599	46488	47762	WP_000457541.1_DNA_polymerase_V_subunit_UmuC	+
pSLT	78150	78349	78078	78459	pseudo_pseudo	-
pSLT	91950	92149	91997	92164	WP_001691570.1_DNA_replication_protein	-
pCol1B9	200	549	455	1486	WP_000907875.1_replication_initiation_protein_PK-repZ	+
pCol1B9	1350	1599	455	1486	WP_000907875.1_replication_initiation_protein_PK-repZ	+
pCol1B9	8000	8199	6669	8015	WP_001132021.1_hypothetical_protein	+
pCol1B9	30000	30249	30112	30366	WP_000774889.1_hypothetical_protein	-
pCol1B9	33800	33949	33969	34301	WP_001281051.1_hypothetical_protein	+
pCol1B9	65800	65949	66007	66591	WP_000977522.1_conjugal_transfer_protein_TraG	-
pCol1B9	86250	86649	85922	86158	WP_000483804.1_conjugal_transfer_protein_TraA	-
pRSF1010	5350	5649	3338	5467	WP_001395566.1_mobilization_protein_A	-
pRSF1010	7200	7449	7015	7851	WP_000480968.1_aminoglycoside_phosphotransferase_APH(6)-I	-

S. enterica	Peak start	Peak end	Map position		Gene description	Strand
StdF binding peaks						
SL1344	23050	23349	23335	24039	WP_000738617.1_hypothetical_protein	+
SL1344	33800	33999	33364	34368	WP_001247395.1_LysR_family_transcriptional_regulator	+
SL1344	256950	257199	257144	257701	WP_000622423.1_ribosome-recycling_factor_frr	+
SL1344	288850	289049	288235	288801	WP_001051726.1_D-glycero-beta-D-manno-heptose-1,7-bisphosphate_7-phosphatase	+
SL1344	386250	386399	385794	386294	WP_001539242.1_hypothetical_protein	+
SL1344	388200	388349	388268	389011	WP_000081378.1_transcriptional_regulator	+
SL1344	492150	492299	491265	492758	WP_000595924.1_hypothetical_protein	+
SL1344	531400	531499	528735	531884	WP_001132508.1_multidrug_efflux_RND_transporter_permease_subunit	-
SL1344	555450	555549	555572	556366	WP_000421859.1_hypothetical_protein	-
SL1344	692350	692549	692403	692612	WP_000034826.1_cold-shock_protein	+
SL1344	701400	701599	701433	703334	WP_000828700.1_penicillin-binding_protein_2	-
SL1344	749450	749549	748945	750351	WP_001258803.1_chitoporin_chiP	+
SL1344	768650	769099	766424	769108	WP_000997487.1_two-component_system_sensor_histidine_kinase_KdpD	-
SL1344	792600	792699	791133	793082	WP_000367426.1_glycosyl_transferase	+
SL1344	822850	822999	823028	824080	WP_001109234.1_phospho-2-dehydro-3-deoxyheptonate_aldolase	+
SL1344	840250	840399	839835	840851	WP_001265465.1_UDP-glucose_4-epimerase	-
SL1344	847500	847699	847453	848271	WP_000127031.1_pyridoxal_phosphate_phosphatase	-
SL1344	855000	855149	854223	855908	WP_001115209.1_urocanate_hydratase	+
SL1344	873350	873549	873495	874202	WP_000529073.1_membrane_protein	+
SL1344	874500	874599	874235	874834	WP_000512322.1_membrane_protein	+
SL1344	898600	898799	898795	899310	WP_000716763.1_outer_membrane_protease_ompX	+
SL1344	913600	913999	913046	913945	WP_000576947.1_pyruvate_formate_lyase_activating_enzyme	-
SL1344	931500	931699	931284	932174	WP_000545327.1_LysR_family_transcriptional_regulator	-
SL1344	965500	965649	965604	966605	WP_000566344.1_threonine_aldolase	-
SL1344	974950	975049	974052	975002	WP_001519667.1_virulence_protein_VirK	-

SL1344	1033900	1034099	1033973	1034776	WP_001154025.1_S-adenosyl-L-methionine-dependent_methyltransferase	+
SL1344	1068750	1069049	1068940	1069257	WP_010989004.1_hypothetical_protein	+
SL1344	1086550	1086799	1086605	1087138	WP_000877926.1_Superoxide_dismutase_[Cu-Zn]_1	-
SL1344	1089850	1089999	1089344	1092694	WP_000033415.1_host_specificity_protein_J	+
SL1344	1096850	1097199	1096293	1097261	WP_001533476.1_secreted_effector_protein_SseI	+
SL1344	1099650	1099949	1099845	1100036	WP_000497441.1_DNA-damage-inducible_protein_I	-
SL1344	1112600	1112749	1111507	1113147	WP_000433414.1_paraquat-inducible_protein_B	+
SL1344	1115900	1116099	1114817	1116577	WP_000156453.1_lon_protease	-
SL1344	1135700	1135949	1134553	1136238	WP_001166946.1_inositol_phosphate_phosphatase_SopB_sopB	-
SL1344	1172850	1172999	1172764	1174260	WP_000628082.1_acetylneuraminate_ABC_transporter	-
SL1344	1189450	1189799	1189629	1191116	WP_000976323.1_hypothetical_protein	+
SL1344	1270000	1270099	1269559	1270788	WP_000359410.1_peptidase_T	+
SL1344	1289850	1289999	1289672	1289843	pseudo_pseudo	-
SL1344	1290250	1290399	1290067	1290143	tRNA-Arg_tRNA-Arg	+
SL1344	1309800	1309999	1309991	1310239	WP_000512149.1_membrane_protein	+
SL1344	1368100	1368299	1366940	1368163	WP_000905564.1_O-antigen_polymerase	-
SL1344	1446800	1446949	1446884	1447132	WP_001574409.1_type_III_secretion_system_protein_SsaI	+
SL1344	1484900	1485149	1483302	1484807	WP_000100911.1_dipeptide_and_tripeptide_permease_A_tppB	-
SL1344	1550650	1551149	1550924	1551019	WP_000901531.1_membrane_protein	-
SL1344	1577150	1577299	1576636	1578126	WP_001519903.1_PhoPQ-regulated_protein	-
SL1344	1601700	1602049	1602034	1602249	WP_000495700.1_protein_bdm	+
SL1344	1649700	1649799	1649034	1649984	WP_001122927.1_secreted_effector_protein_SifB_sifB	+
SL1344	1665600	1665799	1664695	1665897	WP_000848076.1_L-lactate_oxidase	-
SL1344	1706800	1706999	1707001	1707984	WP_000387373.1_zinc_transporter_ZntB_zntB	-
SL1344	1734300	1734599	1733034	1734575	WP_001235493.1_transcriptional_regulator	-
SL1344	1764850	1765049	1764839	1764967	WP_001138871.1_hypothetical_protein	-
SL1344	1893400	1893699	1893337	1893480	WP_001537930.1_hypothetical_protein	-

SL1344	1894500	1895149	1894788	1895027	WP_001236777.1_hypothetical_protein	+
SL1344	1902100	1902249	1902180	1903463	WP_001207294.1_membrane_protein	+
SL1344	1935050	1935299	1935189	1937000	WP_001069113.1_phosphogluconate_dehydratase	-
SL1344	1979600	1979999	1979284	1979625	WP_001518146.1_flagellar_transcriptional_regulator_FlhD	-
SL1344	1980500	1980699	1980421	1980849	WP_000122606.1_universal_stress_protein_C	+
SL1344	1983150	1983349	1983289	1983471	WP_000202368.1_sugar_ABC_transporter_permease	-
SL1344	1995500	1995599	1993986	1995818	WP_001289464.1_UvrABC_system_protein_C_uvrC	-
SL1344	2064750	2064949	2064878	2065501	WP_000188927.1_hypothetical_protein	-
SL1344	2072150	2072299	2072244	2072939	WP_001020636.1_transcriptional_regulator	+
SL1344	2079450	2079549	2078785	2079774	WP_000532847.1_integrase	+
SL1344	2140300	2140399	2139285	2141633	WP_000703998.1_E3_ubiquitin--protein_ligase	+
SL1344	2165100	2165549	2165334	2166335	WP_000908622.1_abequosyltransferase_RfbV	-
SL1344	2167550	2167749	2167714	2168613	WP_000143399.1_CDP-abequose_synthase	-
SL1344	2291900	2292149	2291982	2292869	WP_000022915.1_phosphoserine_phosphatase	+
SL1344	2292650	2292999	2292902	2294224	WP_000023807.1_hypothetical_protein	+
SL1344	2332500	2332899	2332550	2333077	WP_001215679.1_tail_protein	-
SL1344	2335700	2336049	2335540	2336871	WP_000894640.1_NTPase	+
SL1344	2336700	2336949	2336900	2337265	WP_001204799.1_antitermination_protein_Q	-
SL1344	2357100	2357399	2356870	2357361	WP_000228070.1_ferredoxin	-
SL1344	2388000	2388099	2387952	2389580	WP_000857292.1_sn-glycerol-3-phosphate_dehydrogenase_subunit_A_glpA	+
SL1344	2450350	2450449	2449296	2450816	WP_000117932.1_hypothetical_protein	+
SL1344	2508950	2509149	2508217	2509491	WP_031602386.1_ABC_transporter_substrate-binding_protein	-
SL1344	2509400	2509649	2509454	2509672	WP_000279836.1_hypothetical_protein	-
SL1344	2595700	2596049	2595410	2596537	WP_001277825.1_succinyl-diaminopimelate_desuccinylase	+
SL1344	2618700	2618899	2618611	2618748	WP_001540598.1_hypothetical_protein	-
SL1344	2623700	2623799	2623795	2625144	WP_000953165.1_exodeoxyribonuclease_7_large_subunit_xseA	+
SL1344	2681950	2682199	2682152	2682955	WP_000553467.1_inositol-1-monophosphatase	+

SL1344	2684350	2684649	2684160	2685203	WP_000985204.1_sulfite_reductase_subunit_alpha	+
SL1344	2695850	2696249	2694348	2695892	WP_001187150.1_transcriptional_regulator	+
SL1344	2705450	2705599	2703891	2705276	WP_001542313.1_two-component_system_sensor_histidine_kinase	-
SL1344	2726750	2727099	2726717	2728186	WP_001526392.1_type_III_secretion_system_protein	+
SL1344	2727950	2728299	2726717	2728186	WP_001526392.1_type_III_secretion_system_protein	+
SL1344	2759600	2759799	2759753	2760316	WP_000211410.1_antirepressor	-
SL1344	2802300	2802399	2802529	2805102	WP_001235094.1_chaperone_protein_ClpB	-
SL1344	2807850	2807999	2807072	2807809	WP_000197660.1_outer_membrane_protein_assembly_factor_BamD	+
SL1344	2821400	2821549	2820345	2822183	partial;pseudo_partial;pseudo	+
SL1344	2855200	2855499	2855616	2855834	WP_000980498.1_hypothetical_protein	-
SL1344	2879150	2879299	2879070	2879447	WP_000698372.1_hypothetical_protein	-
SL1344	2888750	2889049	2888519	2889730	WP_000834152.1_hypothetical_protein	+
SL1344	2924550	2924699	2924046	2925920	WP_001521074.1_membrane_protein	+
SL1344	2929950	2930199	2929642	2929908	WP_001224043.1_transposase	-
SL1344	2935450	2935649	2935199	2935738	WP_000388997.1_repressor_of_phase_1_flagellin_gene	-
SL1344	2951200	2951399	2950949	2951878	WP_000178733.1_VirG_localization_protein_VirK	+
SL1344	3041900	3042049	3042432	3044093	WP_001120085.1_transcriptional_regulator_HilA	+
SL1344	3051500	3051699	3051690	3053471	WP_000245788.1_cell_invasion_protein_SipB	-
SL1344	3089650	3089999	3089110	3090243	WP_001272632.1_murein_hydrolase_activator_NlpD_nlpD	-
SL1344	3117550	3117699	3118020	3118691	WP_001199961.1_7-carboxy-7-deazaguanine_synthase	-
SL1344	3138150	3138599	3138274	3138486	WP_000988723.1_hypothetical_protein	-
SL1344	3156800	3156899	3156998	3157225	WP_000750393.1_lipoprotein	-
SL1344	3205450	3205999	3205639	3206475	WP_000503692.1_cobalt_transporter_yohM	+
SL1344	3208000	3213699	3209943	3212432	WP_000705483.1_fimbrial_usher_protein	-
SL1344	3214050	3214299	3213855	3214487	WP_000835265.1_hypothetical_protein	-
SL1344	3242800	3243099	3243022	3243618	WP_001520956.1_5-formyltetrahydrofolate_cyclo-ligase	+
SL1344	3259750	3260099	3259380	3260138	WP_000701830.1_metalloprotease	+

SL1344	3338700	3338899	3338755	3339159	WP_000665657.1_hypothetical_protein	+
SL1344	3383050	3383149	3383264	3384697	WP_000867682.1_bifunctional_protein_HldE	-
SL1344	3469300	3469599	3468958	3469842	WP_000802069.1_lipoprotein_NlpI	-
SL1344	3479200	3479399	3479208	3479284	tRNA-Met_tRNA-Met	-
SL1344	3511750	3511899	3511909	3514245	WP_000809815.1_aerobic_respiration_control_sensor_protein_ArcB	-
SL1344	3522500	3522749	3522035	3523138	WP_000184315.1_hypothetical_protein	+
SL1344	3532700	3532849	3532871	3533665	WP_000505557.1_hypothetical_protein	-
SL1344	3552600	3552799	3551420	3553387	WP_000510913.1_p-hydroxybenzoic_acid_efflux_pump_subunit_AaeB	-
SL1344	3578450	3578749	3578064	3578948	WP_000642611.1_methyltransferase	+
SL1344	3597800	3598349	3597162	3598286	WP_000124529.1_hypothetical_protein	-
SL1344	3677800	3678099	3678085	3679704	WP_001265689.1_phosphoenolpyruvate_carboxykinase_[ATP]	+
SL1344	3685250	3685449	3685412	3685639	WP_001160955.1_ferrous_iron_transporter_A_feoA	+
SL1344	3743600	3743849	3743652	3744068	WP_000826041.1_outer_membrane_protein	+
SL1344	3758150	3758349	3758349	3759404	WP_001081707.1_cell_division_protein_FtsX_ftsX	-
SL1344	3768750	3768999	3768246	3768911	WP_000187489.1_membrane_protein	+
SL1344	3829550	3829749	3829506	3829613	WP_001669334.1_hypothetical_protein	-
SL1344	3837250	3837399	3835747	3837354	WP_000028047.1_peptide_ABC_transporter_substrate-binding_protein	-
SL1344	3853150	3853399	3851635	3853968	WP_000148453.1_biotin_sulfoxide_reductase	-
SL1344	3873850	3873999	3871846	3873873	WP_000761305.1_alpha-amylase_malS	+
SL1344	3962350	3962649	3962566	3963957	WP_000115428.1_xanthine_permease	+
SL1344	3974900	3975149	3975383	3976000	WP_000984806.1_hypothetical_protein	+
SL1344	4045300	4045849	4044666	4045850	WP_001230254.1_trimethylamine_N-oxide_reductase_cytochrome_C_subunit	-
SL1344	4126800	4126899	4126828	4126904	tRNA-Asp_tRNA-Asp	+
SL1344	4146800	4147099	4147129	4148388	WP_001054532.1_transcription_termination_factor_Rho_rho	+
SL1344	4167550	4167899	4167912	4170458	WP_000281718.1_adenylate_cyclase_cyaA	+
SL1344	4227200	4227649	4227569	4230355	WP_000249972.1_DNA_polymerase_I	+
SL1344	4252950	4253199	4253044	4254285	WP_000870946.1_sulfoquinovose_isomerase	-

SL1344	4283600	4283849	4283840	4283965	WP_000183584.1_hypothetical_protein	+
SL1344	4284650	4285049	4284831	4286138	WP_000566800.1_membrane_protein	-
SL1344	4336050	4336299	4335722	4336720	WP_000406820.1_membrane_protein	-
SL1344	4407550	4407949	4407263	4407853	WP_000940092.1_hypothetical_protein	+
SL1344	4415650	4415949	4416006	4417559	16S_ribosomal_RNA_16S_ribosomal_RNA	+
SL1344	4501850	4502049	4501156	4502475	WP_001541306.1_ABC_transporter	+
SL1344	4504850	4505049	4503766	4520445	WP_000527217.1_membrane_protein	+
SL1344	4523400	4523549	4523673	4525274	WP_000083640.1_hypothetical_protein	+
SL1344	4582500	4582799	4582602	4582823	WP_001576552.1_hypothetical_protein	-
SL1344	4595050	4595299	4593838	4595079	WP_001670701.1_transporter	-
SL1344	4607600	4607749	4606676	4607653	WP_000004794.1_elongation_factor_P--(R)-beta-lysine_ligase	+
SL1344	4642350	4642549	4642267	4642596	WP_000586836.1_transporter	-
SL1344	4797100	4797299	4796855	4797556	WP_001237610.1_membrane_protein	-
SL1344	4838700	4838799	4838563	4839336	WP_000119016.1_deoxyribonuclease	+
pSLT	12650	12849	11189	14011	WP_001007087.1_conjugal_transfer_protein_TraG	-
pSLT	29800	30099	29946	30512	WP_000399768.1_conjugal_transfer_protein_TraE	-
pSLT	33100	33249	33259	33729	WP_014344446.1_lytic_transglycosylase	+
pSLT	34400	34599	34589	34858	WP_001677523.1_protein_32_protein_of_plasmid	-
pSLT	41300	41449	41218	41652	WP_001200150.1_hypothetical_protein	-
pSLT	46000	47699	46488	47762	WP_000457541.1_DNA_polymerase_V_subunit_UmuC	+
pSLT	48000	48449	47844	48818	WP_000064272.1_chromosome_partitioning_protein_ParB	-
pSLT	48900	49149	48818	50023	WP_000427676.1_chromosome_partitioning_protein_ParA	-
pSLT	78750	78899	79178	79480	WP_000979451.1_hypothetical_protein	+
pSLT	91950	92149	91997	92164	WP_001691570.1_DNA_replication_protein	-
pCol1B9	1600	1799	455	1486	WP_000907875.1_replication_initiation_protein_PK-repZ	+
pCol1B9	15300	15499	15468	16442	WP_001217836.1_hypothetical_protein	+
pCol1B9	32000	34899	32467	33315	WP_001077019.1_hypothetical_protein	+

pCol1B9	35150	35449	34396	37011	WP_000128933.1_hypothetical_protein	+
pCol1B9	44400	44599	43906	44568	WP_000653334.1_ethanolamine_utilization_protein_EutE	-
pCol1B9	65800	65949	66007	66591	WP_000977522.1_conjugal_transfer_protein_TraG	-
pCol1B9	72300	72599	71003	72295	WP_001417545.1_Shufflon_protein_A'	-
pCol1B9	75050	75299	75248	76801	WP_000362202.1_ATP-binding_protein	-
pRSF1010	5300	5749	5666	5950	WP_000238497.1_mobilization_protein_C	+