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Analyse fonctionnelle des fimbriae de type chaperon-placier chez *Salmonella enterica* sérovar Typhi

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Cette thèse intitulée

Analyse fonctionnelle des fimbriae de type chaperon-placier de *Salmonella enterica* sérovar Typhi

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Résumé

Salmonella enterica sérovar Typhi est une bactérie pathogène humain-spécifique et l'agent étiologique de la fièvre typhoïde. Parmi ses facteurs de virulence, il y a 14 systèmes d'adhésion putatifs nommés fimbriae qui ont été identifiés dans le génome de *S.* Typhi. Les fimbriae sont regroupés en opérons qui codent pour des structures protéiques extracellulaires, pour une machinerie de sécrétion et d'assemblage et parfois pour des régulateurs. Ceux-ci sont peu exprimés en conditions de laboratoire et peu étudiés chez *S.* Typhi. Parmi les 14 fimbriae de *S*. Typhi, 12 appartiennent à la classe des chaperon-placier, c'est-à-dire qu'ils possèdent un chaperon et un placier qui leur sont dédiés pour la formation de la structure fimbriaire. Je crois que ces fimbriae sont importants pour la pathogenèse de *S*. Typhi. Le but de ce projet est l'analyse fonctionnelle des fimbriae de type chaperon-placier chez *S.* Typhi. Pour ce faire, j'ai voulu établir une caractérisation générale des 12 fimbriae de type chaperon-placier, puis j'ai concentré l'étude sur la régulation de 2 de ces fimbriae, c'est-à-dire Fim et Std.

La caractérisation générale des fimbriae de type chaperon-placier consistait à déterminer l'expression des promoteurs fimbriaires lors de la croissance en différentes conditions de culture mimant l'infection, à déterminer la présence et la morphologie des fimbriae à la surface de la bactérie et à évaluer l'effet des fimbriae sur la pathogenèse de *S.* Typhi (formation de biofilm, interactions avec les cellules de l'hôte et motilité bactérienne). L'expression maximale des fimbriae a été obtenue principalement en milieu minimal. J'ai observé pour la première fois 6 des 12 fimbriae par microscopie électronique à transmission. Chaque fimbria présentait des effets sur au moins une étape testée sur la pathogénèse. La régulation de *std* et *fim* a été étudiée en déterminant le rôle de régulateurs globaux et par criblage d'une banque de mutants par insertion de transposon. Principalement, j'ai découvert que le promoteur *std* était activé par Crp, responsable de la répression catabolique, tandis que *fim* voit son expression modulée par la chaîne de transport d'électrons (Ndh) et des perturbations de l'enveloppe (OmpR). Finalement, nos résultats démontrent que les fimbriae de type chaperon-placier sont importants pour la

pathogenèse de *S.* Typhi et que deux de ceux-ci sont régulés par des signaux environnementaux importants rencontrés par la bactérie lors de l'infection.

Mots-clés : *S.* Typhi, fimbriae, chaperon-placier, Fim, Std, régulation, pathogenèse.

Abstract

Salmonella enterica serovar Typhi is a human-specific pathogenic bacteria and the etiologic agent of typhoid fever. Among its virulence factors, there are 14 putative adhesion systems named fimbriae identified in the *S.* Typhi genome. Each fimbria is clustered in an operon that encodes for extracellular proteinaceous structures, for the secretion and assembly machinery and sometime for regulators. Fimbrial genes are poorly expressed under laboratory conditions, with few studied in *S.* Typhi. Among the 14 fimbriae, 12 belong to the chaperone-usher class, where each one encodes a dedicated chaperone and usher that form the fimbrial structure. I propose that fimbriae are important for *S.* Typhi pathogenesis. The aim of this project is the functional analysis of all the chaperone-usher fimbriae of *S.* Typhi. My goals were to establish a general characterization of the 12 chaperone-usher fimbriae, and to study specifically the regulation of 2 fimbriae, Fim and Std.

The general characterization of chaperone-usher fimbriae includes the determination of the expression of fimbrial promoters in different growth conditions mimicking infection, the observation of the presence and morphology of fimbriae at the bacterial surface, and the evaluation of the role of fimbriae on *S.* Typhi pathogenesis (biofilm formation, host-cells interactions and motility). Fimbrial expression was generally higher when cells were grown in minimal medium. I was able to observe for the first time the presence of 6 out of 12 fimbriae by transmission electron microscopy. Regarding the role of fimbriae in pathogenesis, each fimbria was involved in at least one step. Regulation of *std* and *fim* was studied by evaluating the implication of several general regulators and by screening a transposon-based library. Overall, I discovered that the *std* promoter was activated by Crp, responsible of catabolic repression, and that *fim* was modulated by the activity of the electron transport chain and by envelope perturbations. Finally, my results demonstrated that the chaperone-usher fimbriae are important for *S*. Typhi pathogenesis and two of them are regulated by important environmental signals encountered during bacterial infection.

Keywords: *S.* Typhi, fimbriae, chaperone-usher, Fim, Std, regulation, pathogenesis.

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ADN : acide déoxyribonucléique

Ap : ampicilline

ARN : acide ribonucléique

ARNm : ARN messager

ATP : adénosine triphosphate

cAMP: adénosine monophosphate cyclique

CFU : *colony-forming unit*

Cm : chloramphénicol

DAP: *diaminopimelic acid*

EBP: *enhanced binding protein*

$EIIA^{Glc}$: enzyme IIA^{Glc}

IPTG: Isopropyl-β-thiogalactopyranoside

Kan: kanamycine

Kb : kilobase

LB : Luria-Bertani ou *lysogeny broth*

LPS : lipopolysaccharides

LTTR : Régulateurs transcriptionnels de type LysR

MAM : molécules d'adhésion multivalentes

pb : paire de bases

PEP : phosphoénolpyruvate

PMA : M phorbol 12-myristate 13-acétate

SCV : vacuole contenant *Salmonella*

SEM : erreur standard de la moyenne (*standard error of the mean*)

S. Enteritidis : *Salmonella enterica* sérovar Enteritidis

S. Paratyphi : *Salmonella enterica* sérovar Paratyphi

S. Typhi: *Salmonella enterica* sérovar Typhi

S. Typhimurium: *Salmonella enterica* sérovar Typhimurium

SPI : Îlot de pathogénicité de *Salmonella*

T1SS : système de sécrétion de type I

T3SS : système de sécrétion de type III

T5SS : système de sécrétion de type V

T6SS : système de sécrétion de type VI

TCS: système à deux composantes

TLR : récepteurs de type ‘’Toll’’

''It is not the strongest of the species that

survive, nor the most intelligent, but the

one most responsive to change.''

Charles Darwin

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Chapitre 1 – Revue de littérature

1.1 Historique de *Salmonella*

Les bactéries du genre *Salmonella* ont été identifiées pour la première fois à la fin du 19e siècle par l'épidémiologiste Theobald Smith sous la direction de Dr Daniel Elmer Salmon au « Bureau of Animal Industry » (BAI), aux États-Unis. La bactérie fût alors nommée *Salmonella cholerasuis*, puis renommée *Salmonella enterica* en 1986 (1). Cette espèce fût longtemps considérée comme la seule du genre *Salmonella*, mais une deuxième espèce, *Salmonella bongori*, fût identifiée par comparaison génétique de génomes dans les années 1980. L'espèce *Salmonella enterica* est elle-même classée en 6 sous-espèces : *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* et *indica*. Ces sous-espèces sont ensuite subdivisées en plus de 2600 sérovars (ou sérotypes) observés à ce jour. Les sérovars sont classés par la classification de Kauffman-White selon la présentation de trois antigènes de surface, c'est-à-dire les antigènes O (lipopolysaccharide), F (flagelle) et Vi (capsule). L'antigène O détermine la sous-espèce à laquelle appartient une souche, puis l'antigène F en détermine le sérovar. La capsule n'est retrouvée que chez quelques rares sérovars dont *S*. Typhi (2).

Des sérovars de *Salmonella*, nous dénombrons aujourd'hui plusieurs bactéries pathogènes d'importance clinique présentant deux pathologies bien distinctes. Les bactéries de certains sérovars tels que Typhimurium et Enteritidis causent une infection dite localisée : cette infection est caractérisée par une inflammation au niveau de l'intestin causant une gastro-entérite. Ce type d'infection à *Salmonella* est habituellement pris en charge rapidement par les défenses de l'hôte et la bactérie est rapidement éliminée au niveau de l'intestin. La deuxième présentation clinique est due à une infection systémique où les défenses de l'hôte sont contrecarrées par plusieurs facteurs de la bactérie menant lentement celle-ci à se disséminer vers les autres organes de l'hôte. Cette deuxième manifestation est retrouvée dans le cas d'une infection par les sérovars Typhi, Paratyphi et Gallinarum (3).

Une deuxième différence majeure entre sérovars est le spectre d'hôte de ces bactéries pathogènes : certains sérovars peuvent infecter une large variété d'hôtes, tandis que certains sérovars sont hôte-spécifiques. Majoritairement, les sérovars hôte-spécifiques causent une infection systémique, tandis que les sérovars à large spectre d'hôte cause une gastro-entérite (3).

Salmonella enterica sérovar Typhi (*S.* Typhi) est l'agent étiologique de la fièvre typhoïde. Cette pathologie est retrouvée dans les pays en voie de développement, particulièrement dans les pays ayant une problématique au niveau de l'eau potable et des installations sanitaires (4). Cette bactérie humain-spécifique est transférée d'un hôte à l'autre par contact avec de l'eau ou de la nourriture contaminées par les fèces ou l'urine d'un porteur. De ce fait, la fièvre typhoïde est retrouvée chez près de 17 millions de personnes par année, engendrant environ 200 000 morts par année. Les signes et symptômes de la fièvre typhoïde comprennent une fièvre prolongée, des malaises, une perte d'appétit, des céphalées, une constipation ou une diarrhée, des taches rosées sur la poitrine, ainsi qu'une augmentation de la taille de la rate et du foie (5).

1.2 Particularités génétiques de *Salmonella*

Plusieurs particularités génétiques discriminent chaque sérovar un de l'autre permettant les différences de présentation clinique et de spécificité d'hôte. Parmi ces particularités, nous comptons la présence d'îlots de pathogénicité, de pseudogènes et de prophages dans le génome de ces bactéries, ainsi que la présence de plasmides variés.

Salmonella possède plusieurs îlots de pathogénicité à haut taux de GC supportant un transfert horizontal. *S.* Typhi possède 18 îlots de pathogénicité de *Salmonella* (SPI), dont 11 sont en commun avec *S.* Typhimurium (SPI-1 à -6, SPI-9, SPI-11 à 13 et -16), 4 sont spécifiques à *S.* Typhi (SPI-7, -15, -17 et -18) et 2 (SPI-8 et -10) sont présents chez *S*. Typhi et absents chez *S.* Typhimurium malgré une trace

génétique distincte chez *S*. Typhimurium (6). Parmi ces SPI, certains portent des facteurs de virulence majeurs pour chacune de ces bactéries, tel que SPI-1 et SPI-2 qui codent pour des systèmes de sécrétion de type III (T3SS) respectivement liés à l'invasion et à la survie face aux cellules de l'hôte. SPI-6 porte les opérons fimbriaires de *saf* et *tcf*, ainsi qu'un système de sécrétion de type VI (T6SS), tandis que le SPI-7 porte les gènes nécessaires à la formation de la capsule Vi, SPI-10 porte le fimbria Sef, SPI-11 code pour la toxine typhoïde chez *S.* Typhi et SPI-18 code pour plusieurs gènes importants à l'invasion chez *S.* Typhi (6).

La présence de pseudogènes est une caractéristique majeure de différenciation entre sérovars hôte-spécifiques ou généralistes. Un pseudogène est un gène muté créant la présence d'un codon d'arrêt (STOP) prématuré, la présence d'un changement de cadre de lecture ou la formation d'une protéine tronquée, qui normalement causerait l'inactivation du gène en question. En fonction des sérovars, nous retrouvons plus de 5% du génome qui possède des pseudogènes. Par exemple, 210 pseudogènes sont retrouvés chez *S.* Typhi (CT18 et Ty2), tandis que *S.* Typhimurium (LT2) possède seulement 39 pseudogènes (7). Il est proposé que cette inactivation de gènes crée la spécification vers un hôte de choix, laissant ainsi de côté les fonctions qui n'ont plus besoin d'être remplies chez cet hôte. Parmi les pseudogènes communs entre *S.* Typhi et *S.* Paratyphi A, plusieurs opérons fimbriaires possèdent des gènes tronqués, proposant un rôle important de ces systèmes dans la spécificité d'hôte (8).

Le génome de *Salmonella* possède plusieurs prophages et vestiges de ceux-ci. Ces phages sont porteurs de plusieurs gènes menant à la virulence bactérienne en finalité. Chez *S*. Typhimurium SL1344, il y a 4 prophages : Gifsy-1, -2 et -3 et SopE. *S.* Typhi possède plusieurs éléments venant de prophages. Les différentes souches de *S.* Typhi sont très clonales entre elles, à l'exception de ces insertions phagiques qui créent des différences d'une souche à l'autre (6, 9).

Plusieurs plasmides sont portés par *Salmonella* pour sa virulence ou pour la résistance à certains antibiotiques et à certains métaux. Par exemple, *S.* Typhimurium possède pSLT qui code pour plusieurs

gènes de virulence tels que l'opéron *spv*, impliqué dans la survie à l'intérieur des macrophages de l'hôte, et le fimbriae Pef. Chez *S*. Typhi CT18, pHCM1 est retrouvé et code pour des gènes de résistance à divers antibiotiques et métaux lourds (6).

Une particularité du génome de S. Typhi est qu'il a subi un réarrangement par recombinaison entre différents opérons d'ARN ribosomaux (ARNr) ou différents éléments IS200. Ce réarrangement fait diverger le squelette génomique de *S.* Typhi comparativement à *S.* Typhimurium et est possiblement responsable de différences entre les deux sérovars vu que certains gènes sont régulés différemment selon un contexte spatial et génétique différent (10).

1.3 *Salmonella enterica* sérovar Typhi

1.3.1 Prévalence et cycle d'infection

S. Typhi est une bactérie pathogène humain spécifique et l'agent étiologique de la fièvre typhoïde. Sa voie d'entrée est par le système digestif, un environnement hostile semé d'obstacles. Premièrement, différentes molécules antibactériennes sont présentes directement au niveau de la bouche, puis la bactérie doit vivre la présence d'une forte acidité au niveau de l'estomac. Suite au passage à l'estomac, la bactérie parcours l'intestin grâce au péristaltisme qui s'y produit, mais aussi grâce à ses flagelles jusqu'aux cellules M présentes à la plaque de Peyer au niveau du colon. Ces cellules spécialisées seraient le point d'entrée de *S.* Typhi vers les macrophages présents qui servent de réservoirs pour la multiplication et le déplacement de la bactérie (4). *Salmonella* forme alors un compartiment, nommé vacuole contenant *Salmonella* (SCV ou ''*Salmonella*-containing vacuole''), à l'intérieur des macrophages. *Salmonella* se répand ainsi à travers le corps humain en passant par les différents centres lymphoïdes et se dissémine dans le sang causant ainsi la phase accrue de l'infection (11).

S. Typhi, suite à une infection aiguë, persiste par formation de biofilm à l'intérieur de la vésicule biliaire. Ainsi, l'hôte infecté devient porteur sain, c'est-à-dire qu'il ne présente plus de signes ou symptômes de la fièvre typhoïde. Le porteur aura alors périodiquement des relâches de *S.* Typhi dans ses fèces (12).

1.3.2 Facteurs de virulence de *S.* Typhi

Plusieurs facteurs de virulence ont été identifiés pour *S.* Typhi, communs avec *S.* Typhimurium ou uniques à *S.* Typhi. Certains de ces facteurs expliquent en partie la spécificité d'hôte et la présentation clinique de *S.* Typhi.

S. Typhi produit une toxine de type A_2B_5 nommée toxine typhoïde. Celle-ci est codée par le gène *cdtB* et nécessite l'action d'un complexe de PltA et 5 PltB (ou PltC), des homologues des composantes de la toxine de *Bordetella pertussis*. Les complexes PltAB ou PltAC permettent de livrer CdtB de l'intérieur de la bactérie vers une cellule cible (13). Lorsque CdtB est en complexe avec PltA et PltB, son effet est plus cytotoxique, tandis que le complexe CdtB, PltA et PltC augmente la leucopénie (14). CdtB reconnaît spécifiquement des glycanes sialylés terminaux des glycoprotéines présentes à la surface des cellules humaines, mais absentes des cellules d'autres animaux. Comparativement à la majorité des toxines AB, la toxine typhoïde ne peut être produite que lorsque la bactérie a formé sa vacuole dans la cellule hôte. Alors, *S*. Typhi forme la toxine et la délivre de la SCV vers l'espace extracellulaire avec l'aide de Rab29, une GTPase de l'hôte, qui semble diriger la vacuole de façon à permettre la sortie de la toxine (13). Le mécanisme de sécrétion de la toxine typhoïde serait similaire au système holine/endolysine des bactériophages. TtsA jouerait un rôle d'endolysine qui permettrait le passage de la toxine à travers la paroi cellulaire, tandis qu'une holine inconnue formerait un pore pour la sortie de TtsA vers le périplasme. Peu est connu par rapport au mécanisme d'exportation de la toxine encore aujourd'hui. Suite à la libération de la toxine typhoïde, les cellules hôtes sont distendues et leur noyau s'élargie jusqu'à deux fois sa taille normale, phénotype typique d'un arrêt du cycle cellulaire entre les phases G2 et M (15). Cet effet cellulaire est dû à l'activité DNase de CdtB qui endommage l'ADN hôte. La régulation de la synthèse de la toxine typhoïde est relativement complexe due aux deux types de complexes qui

peuvent être formés. Pour le complexe comprenant PltB, PhoPQ semble avoir le plus grand effet. Pour le complexe comprenant PltC, SsrAB et OmpR/EnvZ jouent un rôle plus important et l'effet de PhoPQ est quasiment nul (14, 16, 17).

Plusieurs structures de surface au niveau de la membrane externe de l'enveloppe jouent un rôle important pour la virulence chez *S.* Typhi. Parmi celles-ci, il y a les lipopolysaccharides (LPS), la capsule Vi, les systèmes de sécrétion de type III (T3SS), le système de sécrétion de type VI (T6SS), les flagelles, les adhésines non fimbriaires et les fimbriae.

Le LPS de *S.* Typhi est intriqué dans la membrane externe de la bactérie et en sont une des composantes majeures (18). Le LPS est formé de trois parties : le lipide A, les cœurs interne et externe d'oligosaccharides et une chaîne variable de polysaccharides nommée l'antigène O (19). La chaîne de polysaccharides ou antigène O varie en composition et en longueur chez *Salmonella* et permet de déterminer les sous-espèces. Chez *S.* Typhi, les antigènes O une grande hétérogénéité par rapport à leur longueur selon l'environnement. Entre autres, la longueur de l'antigène O augmente en phase stationnaire lorsque les nutriments se font plus rares et qu'il y a activation de RpoS et RpoN. Les chaînes de polysaccharides des LPS de *S.* Typhi sont relativement courtes, car un régulateur majeur, Wzz, est non-fonctionnel, ce qui diminue la reconnaissance par le TLR4 (20). Les LPS sont essentiels à la virulence pour *S.* Typhi en infection de souris humanisées (hu-SRC-SCID) (21). Les LPS sont masqués en présence de la capsule Vi (22). Chez *S.* Enteritidis, le facteur Dam régule *wzz* par l'intermédiaire de RcsB et PmrA (23).

S. Typhi possède une capsule lui permettant de s'évader du système immunitaire de l'hôte, principalement en prévenant la reconnaissance des LPS par le TLR4 des phagocytes et en limitant le dépôt de l'unité C3 du complément à la surface des bactéries (24). Cette capsule est composée d'exopolysaccharides de groupe 1 : le polysaccharide Vi est un homopolymère d'acide $\alpha(1\rightarrow4)$-2-acétamido-3-*O*-acétyl-2-déoxy-α-D-galacturonique (25). Le locus *viaB* code pour le régulateur TviA, les

gènes *tviB*, *tviC* et *tviE* importants pour la synthèse du polysaccharide et aussi les gènes *vexA-E* impliqués dans le transport du polysaccharide. Le complexe VexA-D est un transporteur de type ABC (*ATP-binding cassette transporter*) servant à l'export du polysaccharide Vi. Finalement, TviD semble important pour la formation de la capsule, mais son rôle est indéterminé (26). La présence de la capsule semble limiter l'accès aux structures de surface de *Salmonella* comme les T3SS et les fimbriae. La capsule de *S.* Typhi est finement régulée par le régulateur majeur TviA, mais est aussi activée par des systèmes à deux composantes (TCS) tels que OmpR/EnvZ et le système Rcs. La capsule est principalement réprimée par RpoS (22).

Il y a deux T3SS chez *Salmonella*, ceux-ci codés par SPI-1 et -2. Le T3SS-1 est impliqué dans l'invasion des cellules hôtes, tandis que le T3SS-2 est responsable de la survie à l'intérieur des macrophages. Ces machineries complexes de plus de 20 protéines forment un pore à la membrane externe et une aiguille moléculaire qui injecte une série d'effecteurs à la cellule hôte permettant de la remodeler et de la manipuler (27-29). Le T3SS de SPI-1 est régulé principalement par une série de gènes retrouvésdans l'îlot de pathogénicité. HilA est le principal régulateur du T3SS-1 et est activé par HilD et HilC. HilD est réprimé dans certaines conditions par HilE (30, 31). Ces gènes régulateurs spécifiques au T3SS-1 sont affectés par plusieurs régulateurs (Lon, Fur, FimYZ, PhoPQ, FliZ, OmpR/EnvZ, CsrA, SirA/BarA, Lrp, Dam, Mlc et PhoBR) selon les conditions environnementales rencontrées par la bactérie (30, 32, 33). HilA active la synthèse du système de sécrétion, mais aussi *invF* qui permet la synthèse des effecteurs du T3SS-1. Chez *S.* Typhi, l'alarmone (p)ppGpp régulerait conjointement le T3SS-1, la capsule et les flagelles (34). Le T3SS de SPI-2 est régulé principalement par un TCS nommé SsrAB. Le T3SS-2 serait aussi régulé par OmpR et PhoP (35-38). Chez *S.* Typhi, SPI-2 serait membre du régulon LeuO (39, 40).

Le T6SS pourrait être comparé à une seringue moléculaire pour la bactérie. C'est une machinerie de sécrétion très similaire à la queue et la gaine de certains bactériophages, ce qui confère à ce système une force contractile pour injecter ses effecteurs vers la cellule hôte ou autres cellules bactériennes cibles. Chez *S*. Typhi, les gènes codant pour ce système sont retrouvés sur le SPI-6. Parmi ces gènes SciI est pseudogène et représente un homologue de VipB qui forme la partie contractile du T6SS chez *Vibrio*

cholerae (41). Le T6SS semble toutefois fonctionnel et augmente la cytotoxicité vis-à-vis les cellules hôtes (42). Ce système serait régulé principalement par PmrA, le système Rcs et Hfq (42, 43).

Salmonella porte généralement plusieurs flagelles péritriches. Un flagelle est un T3SS modifié avec une extrusion de flagellines formant une longue structure protéique extracellulaire qui active le TLR5 présent sur les monocytes, les cellules dendritiques et les cellules épithéliales. Le rôle des flagelles est principalement lié à la motilité bactérienne et la chimiotaxie, mais ils ont aussi été associés à l'invasion et la survie dans les macrophages (44, 45). Un vaste réseau de régulation chapeauté par FlhCD est établi pour les flagelles. Les flagelles et le T3SS-1 sont corégulés. Chez *S.* Typhi, TviA régule directement les gènes flagellaires pour diminuer leur transcription et éviter la reconnaissance de la bactérie par TLR5 (46). Les flagelles seraient aussi atténués lorsqu'il y a augmentation de la température d'incubation (47). Comparativement à *S.* Typhimurium qui code deux structures flagellaires (FliC et FljB), *S.* Typhi code seulement pour *fliC*. Cependant, des variants antigéniques (H*j* et H*z66*) sont retrouvés chez certaines souches de *S*. Typhi, entre autres originaires d'Indonésie. Ceux-ci produisent le même type de réaction au TLR5 que le variant majeur H*d*, mais varient quant à leur structure flagellaire, leur motilité et leur immunogénicité (45).

Les adhésines dites non-fimbriaires incluent les adhésines sécrétées par un T1SS, T5SS, PagC, STY0351 et STY1980. Deux adhésines sont sécrétées par un T1SS, c'est-à-dire SiiE et BapA. Cependant, SiiE est un pseudogène suggérant une perte de fonction chez *S.* Typhi. BapA serait important pour l'autoaggrégation et la formation de biofilm chez *S.* Enteritidis (48). Il serait régulé par CsgD principalement (49). Les adhésines non-fimbriaires sécrétées par un T5SS sont dites auto-transportées. ShdA et MisL sont codés par des pseudogènes chez *S.* Typhi alors qu'ils lient la fibronectine chez d'autres sérovars (50). SadA et YaiU semblent fonctionnels. Pour ce qui est des autres adhésines non-fimbriaires, PagC et STY0351 semblent régulées par PhoPQ et seraient présentes lors de la pathogenèse de *S.* Typhi (36, 51). Le rôle de STY1980 n'est pas établi, mais la protéine est similaire aux molécules d'adhésion multivalentes (MAM) présentes chez certaines *E. coli* pathogènes (52).

1.4 Fimbriae de *Salmonella enterica*

Chaque sérovar de *Salmonella enterica* possède un répertoire particulier de fimbriae lui conférant une spécificité d'adhésion aux surfaces biotiques et abiotiques. *S*. Typhi a une combinaison unique de 14 fimbriae qui pourrait expliquer sa spécialisation pour l'hôte humain, ainsi que sa pathogenèse. Trois types de fimbriae sont présents chez *Salmonella* et nommés selon leur mode d'assemblage, c'est-à-dire les pili de type IVb, les fimbriae curli et les fimbriae de type chaperon-placier. Dans la section 1.4.1 (article 1), ces trois modes d'assemblage sont détaillés en plus de fournir une distribution et une occurrence des fimbriae à travers les différents sérovars de *Salmonella*. Les aspects généraux de la régulation des fimbriae chez *Salmonella* sont ensuite abordés dans une revue sur la régulation du fimbria de type 1 de *S.* Typhimurium, c'est-à-dire le fimbria Fim. Finalement, l'utilisation des fimbriae comme moyens de détection pour *Salmonella* ou comme possibles cibles thérapeutiques contre cette bactérie est discutée à la fin de la section 1.4.1. L'article 1 est un chapitre de livre proposé à Intech et soumis à un processus de révision par les pairs.

Article 1: Dufresne, K. D., F. (2017). "*Salmonella* Fimbriae: what is the clue to their hairdo?" in Current Topics in Salmonella and Salmonellosis, ed M. Mares (Rijeka: InTech.), 59–79.

Contribution des auteurs:

J'ai réalisé les figures et ai rédigé l'article. France Daigle a réalisé les tableaux et aidé à la rédaction. Tous les auteurs ont révisé l'article. France Daigle a fourni le support financier.

1.4.1 Article 1: *Salmonella* Fimbriae: What is the Clue to Their Hairdo?

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1.4.1.1 Abstract

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

Keywords: Fimbriae, adhesion, Chaperone/usher, curli, type IV fimbria, fimbriome, *fim*

1.4.1.2 Introduction

Multiple virulence factors are implicated in *Salmonella* pathogenesis. These factors include type 3 secretion systems (T3SS) encoded in *Salmonella* Pathogenicity Islands (SPI) -1 and -2, other SPIs, flagella, capsule, plasmids and adhesion systems (6, 53). Among those factors, fimbriae represent a major player in pathogenesis and a source of diversity for *Salmonella* serovars. Fimbriae are the most common adhesion systems and are differentially expressed and found in a specific pattern among each serovars (54, 55).

Historically, the first observation of fimbriae was described in 1901 in *Bacillus anthracis* by Hinterberger and Reitman which hypothesized that the filaments were implicated in nutrients acquisition (56). Then, in 1949, Anderson suggested that the filaments were artefacts due to sample preparation for electron microscopy (57). However, many other studies contradicted Anderson and confirmed the presence of non-flagellar appendages on the bacterial surface. In 1950, Houwink and Van Iterson observed the appendages and described them as shorter and more rigid filaments than the flagella from *Escherichia coli* and suggested that the fibres were implicated in attachment to surface (58). The name fimbria (Latin word for fibres) was suggested in 1955 by Duguid et al. to describe the filamentous structures (58, 59). The term fimbria is preferable to use to describe non-flagellar filaments than pili, which is used to designate structures implicated in conjugation (60, 61). In 1966, Duguid et al. classified fimbriae in seven types (types 1 to 6 and F) according to the morphology and haemagglutination patterns. However, another classification, based on serology, better predicted genetic relatedness of fimbrial antigens. Nowadays, fimbriae are designated by the mode of assembly of the fibril (59).

A specific fimbrial gene cluster (FGC) encodes for the structural, assembly and sometime regulatory proteins required for the production of the filamentous adhesive appendage on the bacterial surface. FGCs are usually composed of four to up to fifteen genes (61, 62). An average of 12 FGCs by strains was observed in *S. enterica*. Though all *Salmonella* genomes harbour multiple FGCs, very few have been characterised to date. Most fimbriae are poorly expressed under laboratory conditions and

the functional redundancy complicates their studies (61). However, fimbriae are implicated during infection and in a variety of other roles, like biofilm formation, seroconversion, haemagglutination, cellular invasion and macrophage interactions (6, 58, 63-67). In mice model, *S.* Typhimurium fimbriae demonstrate a role in intestinal cells attachment, caecum colonisation and persistence in gut (68-70). Moreover, fimbriae are important determinants of host adaptation by *Salmonella* (71).

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

1.4.1.3 Fimbrial biogenesis pathways

Three pathways for fimbrial assembly exist in *Salmonella*, the chaperone/usher (CU), the nucleation/precipitation and the type IV pathway (72). Fimbriae of the CU pathway employ dedicated chaperones and ushers for the fimbrial assembly. The nucleation/precipitation pathway forms an aggregative fibre by precipitation of the subunits in presence of the nucleator in the extracellular environment. Finally, the type IV fimbrial pathway uses complex machinery for the fimbriae formation and needs ATP to drive the assembly reaction. Furthermore, the type IV fimbriae can be retracted and diassembled (72).

The three pathways produce quite different fimbriae. CU fimbriae have the classic fimbrial shape with the repetition of major subunits emerging from the usher inserted in the outer membrane. The major subunits can be accompanied by minor subunits and/or adhesins (59). The fimbriae produced by the nucleation/precipitation pathway have an aggregated shape, due to the precipitation of major subunits together. This kind of fimbriae is highly stable and hardly depolymerised (73). The type IV fimbriae anchor in the inner membrane and are prolonged by the repetition of the major subunit (pilin)

through the periplasm and the outer membrane reaching the extracellular medium (74). Here, the three fimbrial assembly mechanisms will be detailed.

1.4.1.3.1 Chaperone/usher pathway

The CU fimbriae represent the largest and most diversified class of adhesion systems (75, 76). Multiple CU fimbriae are present in *Salmonella* suggesting a functional redundancy (9, 74). The assembly is characterised by an interaction between the subunits, a periplasmic chaperone, and an outer membrane usher in order to form a mature fibre (Figure 1) (77). Each fimbriae produced by this pathway have their own unique and specific chaperone and usher (62). Usher sequence is a good discrimination tool and is used to subdivide the CU fimbriae into six phylogenetic clades (α, κ, π, σ, γ, β) (9, 61).

The biogenesis of the CU fimbriae begins with the production of the subunits in the cytoplasm and their export through the inner membrane by the general secretory pathway (GSP) (74, 77, 78). It consists in a post-translational translocation implying the SecYEG complex and SecDF/YajC proteins. When the pre-protein is produced, it can be targeted directly to the accessory factor SecA or transported to SecA by the general chaperone SecB. Then, SecA catalyzes the hydrolysis of ATP to energize the translocation through SecYEG. Use of ATP, in combination with proton-motive force, triggers the transport of the pre-protein to the periplasm. During the translocation across the inner membrane, the N-terminal signal peptide is cleaved by periplasmic peptidases (77, 79). To prevent early folding of the subunits, the fimbrial chaperone instantly forms a complex with the translocated subunit in the periplasm (80).

Fimbrial chaperone shares conserved structural features with the general periplasmic chaperones (80). They are formed of two β-sheet domains oriented to produce a L-shaped molecule and, together form a β-barrel. Each domain has an immunoglobulin-like fold and is composed of seven

primary β-strands (80-82). Hydrophobic residues are alternated in the seven strands, facing the internal part of the barrel. These residues form the hydrophobic core of the domain that is implicated in the binding of the subunit. The fimbrial chaperones have an extended loop that lies at the extremity of one arm of the L-shaped molecule. This loop contains a conserved motif that is involved in the complex formation between the chaperone and subunits (80). The subunit and the chaperone have a similar structure, but the subunit is missing the seventh β-strand of the C-terminal extremity (78). The chaperone transfers the missing β-strand to the subunit to complete its structure: this mechanism is called the donor strand complementation (76). The chaperone preserves the folding energy of the subunit to drive the last steps of the assembly due to lack of energy source (ATP) in the periplasmic space (83). The chaperone also prevents premature fimbrial formation in the periplasm and primes the assembly through the usher (80, 84).

Then, the uncapping of the chaperone by the usher expose the interactive surface of the subunit to the outer membrane usher and assembly of subunits at the surface can occur (83). The transfer of the subunit from the chaperone to the usher happens very rapidly *in vivo*. In absence of the usher *in vitro*, only a slow and inefficient assembly was observed. This suggests that the uncapping of the chaperone is important for the efficiency of mature fimbriae assembly (78, 80). An interaction between the usher and the subunit, and also between the usher and the chaperone is required (81). This triangular interaction is important for the usher to discriminate subunit-loaded from unloaded chaperone (83). Fimbrial usher forms a ring in the outer membrane with a transient twin-pore of 2-3 nm diameter to allow passage of subunits to the extracellular environment (85). The usher catalyses fimbrial polymerisation by involving donor strand exchange where the N-terminal sequence of the subunit is replaced by a short sequence of the last subunit in the polymerized fibril with a zip-in-zip-out mechanism (83). This step is triggered in part by the chaperone required for the strand exchange between the new subunit and the forming fimbria. The quaternary structure of the subunit is achieved when the protein passes through the pore. The final morphology and structure (rigid or flexible), the length (1 to 3 μm) and width (2 to 10 nm) of the fibre of the CU fimbriae depend on the subunits composition and the interactions between subunits (61, 74).

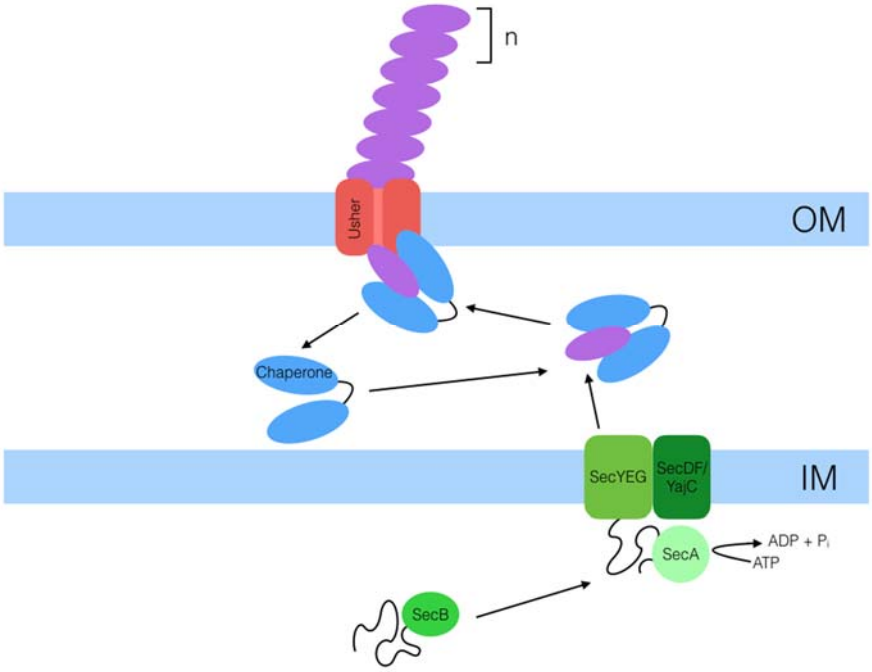

Figure 1. – Chaperone/usher pathway.

The subunit proteins are synthesised in the cytoplasm and translocated through the GSP. When the signal peptide is cleaved from the subunit, the chaperone complements the missing strand of the subunit in a process called donor strand complementation. The energy from the folding of the subunit is preserved by the chaperone. The chaperone drives the subunit to the usher and exchanges the donor strand. The subunit is then translocated by the usher to the extracellular medium and added to other subunits to form the fibril. IM=inner membrane; OM=outer membrane.

1.4.1.3.2 Nucleation/precipitation pathway

Curli fimbriae were initially discovered in *Escherichia coli* and are very conserved among the *Enterobacteriaceae* family, compared to any other types of FGC. The amyloids fibrils are particularly known for their role in biofilm formation and its recognition by the immune system (86). The FGC for curli is named *csg* (curli subunit gene) for *E. coli* and *agf* (thin aggregative fimbriae) for *Salmonella*, but the term *csg* is now commonly used for *Salmonella.* Curli formation depends on two divergent operons, *csgBAC* and *csgDEFG*. The *csgBAC* genes encode for CsgA, the major subunit, CsgB, the nucleator, and CsgC, an oxidoreductase of unknown function. The *csgDEFG* genes encode for the transcription regulator of the operon (CsgD) and for the assembly proteins located in the periplasm (CsgE) or in the outer membrane (CsgG and CsgF) (87).

The curli assembly mechanism is characterised by the exportation of the subunits and their precipitation to each other in the presence of a nucleator that fixes the fibril on the bacterial surface. Exportation of curli proteins also uses the GSP to pass through the inner membrane to the periplasm. Then, the CsgA and CsgB proteins are secreted by the lipoprotein CsgG. CsgG is composed of nine anticodon-binding-domain-like units that form a 36-stranded β-barrel complex that is inserted in the outer membrane. CsgG forms a pore in the outer membrane that permits the passage of the subunits and the nucleator. CsgG is accompanied by the accessory proteins CsgE and CsgF. CsgE is a specificity factor that forms a nonameric adaptor that binds to CsgG and closes the periplasmic space. The presence of CsgE optimizes the uptake of CsgA by CsgG and translocation of CsgA (88). CsgF helps the nucleation activity of CsgB. It was suggested that CsgF has a role in specific localisation and/or chaperoning of the nucleator, so CsgB will reach its full activity. Moreover, CsgF depends on CsgG and CsgE for its stability (89).

Once at the bacterial surface, the nucleator polymerises the subunits together into thin aggregative fimbriae (fibrils). This process happens only in the extracellular environment and requires the presence of the nucleator CsgB to polymerise CsgA into a filament. CsgA proteins fold into an

insoluble cross β-sheet molecules (9). CsgB anchors the curli fimbriae on the surface of the bacterial cell (Figure 2). In *E. coli*, it was observed that CsgB, in addition to its role of nucleator, is also part of the fimbriae with the CsgA subunits. A structurally different fibril made of CsgB subunits can be formed in the absence of CsgA (90). CsgA and CsgB share 30% of sequence identity and have the same predicted length (87). In *E. coli*, interbacterial complementation between a nucleator mutant and a subunit mutant is possible. However, in *Salmonella*, this complementation cannot happen, suggesting that the curli fimbriae are different in their nucleation process. However, the interbacterial complementation was observed in *Salmonella* when a lipopolysaccharide O-antigen mutant was used (91). The nucleation/precipitation pathway is still poorly understood and research is actually performed on the different aspects of the curli fimbrial formation.

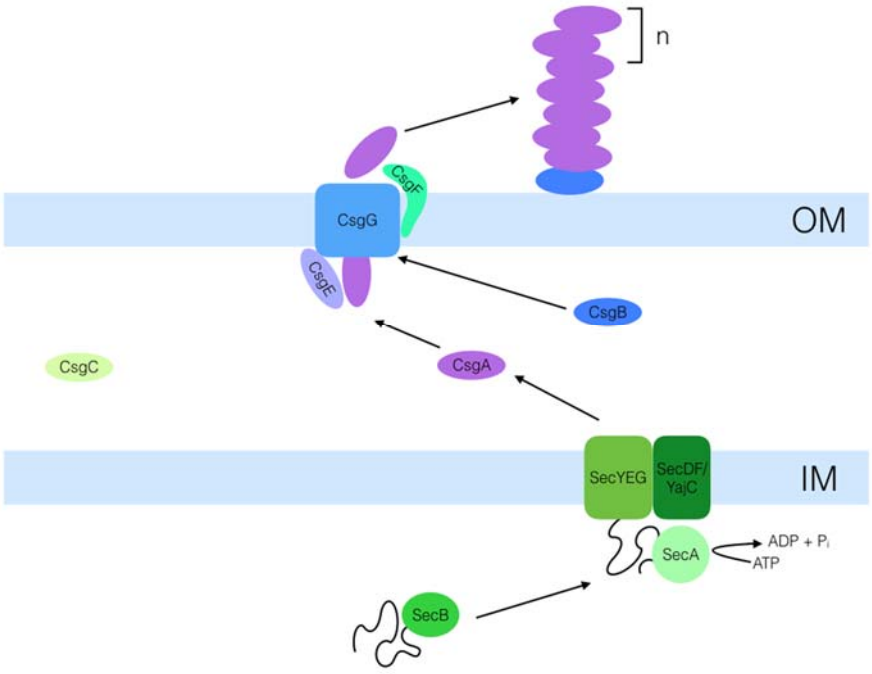

Figure 2. – Nucleation/precipitation pathway.

The subunit CsgA is synthesized in the cytoplasm and translocated by the GSP. CsgA passes through the periplasm and is translocated in the extracellular medium by CsgG, helped by CsgE. The nucleator CsgB is also translocated by CsgG and supported by CsgF for its stability on the bacterial surface. When CsgA is in presence of the nucleator in the extracellular environment, the subunits precipitate in an aggregated fibril. CsgC is an oxidoreductase, but its specific role is still unknown. IM= inner membrane; OM=outer membrane.

1.4.1.3.3 Type IV fimbriae

Type IV fimbriae are usually from 1 to 5 μm long and are composed of repeated subunits of a single pilin. Type IV fimbriae are subdivided in two groups based on homology of the major subunits: type IVa and type IVb fimbriae (9). The difference between the two types is in the length of the peptide sequence and the mature major pilin sequence. Specific assembly mechanisms for type IVb fimbriae from *Salmonella* have yet to be characterised (92).

Type IV fimbriae pathway have the most complex machinery. They form an apparatus, composed of various proteins, that goes through the inner and outer membranes allowing the anchor of the fibre and energy accessibility for fimbrial assembly. The gene cluster also encodes numerous proteins with diverse functions, as the fibril is not only assembled but also disassembled. Type IV fimbriae are frequently compared to the type II secretion system (T2SS) which possesses similar structure and mechanism of assembly. Type IV fimbriae are implicated in adherence and twitching motility (62).

Type IV fimbriae are present in a variety of organisms including human pathogens such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. For *Salmonella*, they are found in *S. bongori*, *S. enterica* serovars Heidelberg, Parathyphi B and Typhi (92). *S. bongori* type IV fimbria is encoded by the *sbe* operon that remains uncharacterised and is located on a plasmid, as well as in *S.* Paratyphi B. While the type IVb gene cluster is located on the chromosome of *S.* Heidelberg and *S.* Typhi (9).

For *S.* Typhi, the PilS subunits are produced in the cytoplasm and translocated to the periplasm by the GSP. In the periplasm, the N-terminal sequence of PilS is cleaved by PilU, a prepilin peptidase (74). The mature pilins are then anchored to the inner membrane on a platform protein and linked together into a fibril (Figure 3) (9, 93). The N-terminal domain of the mature subunits is highly hydrophobic, which permits the PilS proteins to group into a helical structure (73, 92). The pilins are

added one by one, but at three sites simultaneously, each corresponding to a strand to form a three-helix bundle (94). An ATPase inserted in the inner membrane supplies the energy required for the assembly of the type IV fimbriae. The secretin proteins are inserted in the outer membrane and form a channel that permits the passage of the intact pilus through the bacterial surface (9). These proteins form complexes that are then assembled in a cage-like final structure (94). Other proteins are also involved in the assembly/disassembly mechanisms, such as another ATPase dedicated for the disassembly of the fimbriae, lipoproteins of the secretin complex (pilotins), inner membrane proteins or gene products involved in peptidoglycan remodelling to permit the passage of the fibril through the periplasm (73, 94, 95). This assembly pathway is less understood and requires further investigations (94).

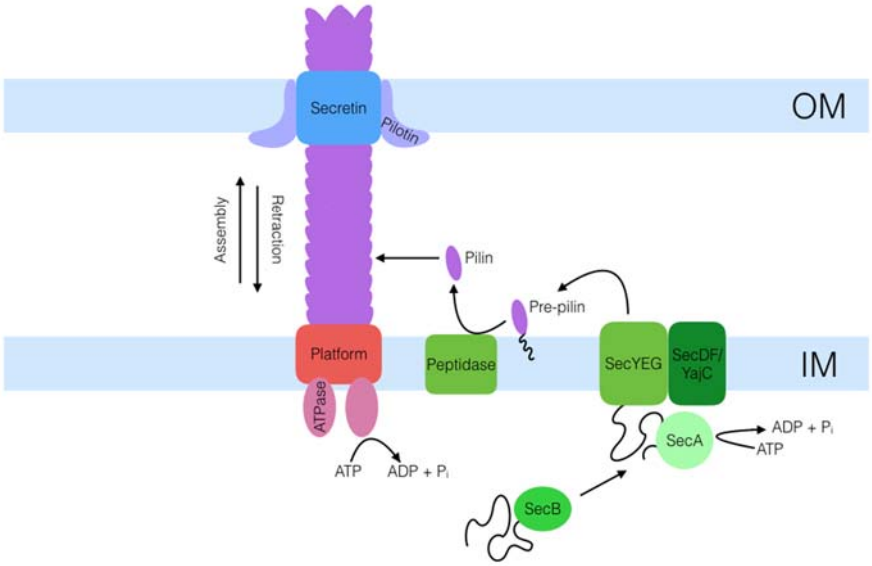

Figure 3. – Type IV pathway.

The prepilins are transported and translocated through the inner membrane (IM) to the periplasm by the GSP. A peptidase cleaves the signal peptide of the pre-pilin and the pilin can be assembled on the platform protein. An ATPase triggers the reaction. The pilins form a three-helix structure that passes through the outer membrane (OM) by a secretin supported by pilotins. The type IV fimbriae can also retract depending of the environmental conditions.

1.4.1.4 Salmonella fimbriome

Each fimbrial pathway described above is present in *Salmonella* creating a great element of genetic diversity. CU fimbriae are the most common fimbriae detected in the *Salmonella* genome. Curli (*csg*) is found in all *Salmonella* genome, whereas only a few serovars have the type IV fimbriae. There are 38 unique FGCs identified so far in 111 sequenced genomes from 34 different serovars (Tableau 1) (96, 97). Each serovar have their own repertoire of FGCs but there are seven FGCs that are highly conserved in most *Salmonella* strains forming the core of *Salmonella* FGCs. Most of the FGCs are sporadic or found only in a few strains constituting the signature of each serovar.

Tableau 1. – Salmonella fimbriome

Fimbriae	CU clade	Prevalence	Distribution
Bcf	γ1	core	absent in IV
Csg	curli	core	all *Salmonella*
Fim	γ1	core	absent in *bongori*
Lpf	γ1	conserved	absent in ID
Mrk	γ4	sporadic	only in Montevideo
Pef	κ	sporadic	only in IA, IC and *bongori*
Peg	γ4	conserved	IB, IC, IIIa, VI, *bongori*
Peh	γ4	sporadic	only in Montevideo
Pil		sporadic	Type IV; ID, IE, *bongori*
Saf	γ3	conserved	ssp. I
Sba	γ4	sporadic	*Bongori*
sbb/sbf	π	sporadic	*Bongori*
sbc/spf	κ	sporadic	IV, VI*, bongori*
Sbs	β	sporadic	II *salamae*
sdc/sas	σ	sporadic	IIIa *arizonae*
sdd /smf	γ1	sporadic	IE, II, IIIa, IV
Sde	γ3	sporadic	Tennessee (IE)
Sdh	γ4	sporadic	IE
sdi/sdf	γ4	sporadic	IIIb *diarizonae*

Fimbriae	CU clade	Prevalence	Distribution
sdj	γ4	sporadic	IIIb *diarizonae*
sdk/sfi	π	sporadic	IIIb, VI
sdl	π	sporadic	IIIb *diarizonae*
sef	γ3	sporadic	IB, D (pseudo)
sib	β	sporadic	VI *indica*
fae/skf	κ	sporadic	IB, IE
ssf	γ4	sporadic	II *salamae*
sta	γ4	sporadic	ID
stb	γ4	core	I, II, IIIb;
stc	γ4	conserved	IA, IB, ID
std	π	core	II, IIIa, missing in Gallinarum
ste	π	conserved	missing in IA, IE
stf	π	conserved	missing in ID, IE
stg	γ1	sporadic	ID, bongori
sth	γ1	core	missing IIIa and IIIb
sti	γ1	conserved	missing in ID
stj	β	sporadic	IA, IE
stk	γ4	sporadic	IE
tcf	α	sporadic	IC, ID, IE

Each *Salmonella* strain contains 5 to 14 different CU fimbriae with an average of 12 fimbriae in *S. enterica*. Representatives from all the six phylogenetic clades are present in *Salmonella* (Tableau 2) (9). The γ-fimbriae constitute the largest clade with 22 FGCs and include the highly conserved FGCs (*bcf, fim* and *sth*) that belong to the clade γ-1. The most diverse clade is γ-4, with the conserved *stb* and *stc* or *peg* (*stc-peg*) and many of the new sporadic FGCs. While the α clade (for alternate CU), also known as class 5 fimbriae, has one FGC, *tcf* which is found in several serovars. The σ clade also had only one FGC representative, *sdc*, that was only found in *S. enterica* subspecies IIIa (*arizonae*).

Tableau 2. – Salmonella fimbrial type

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

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

Most cases of salmonellosis in humans are caused by *S. enterica* ssp. I and many of the sequenced serovars were from ssp. I. Thus, 27 out of the 38 FGCs are found in ssp. I. The ssp. I was divided into 5 classes using previous phylogenetic analysis (96, 97) (Tableau 3). The class IA contains broad host range serovars involved in gastroenteritis, mainly serovar Typhimurium. The class IB is formed by serovar Dublin, Enteritidis, Pullorum and Gallinarum, all sharing similar O-antigens and FGCs. The class IC contains serovar Choleraesuis and Paratyphi C and class ID contains the human specific serovars Typhi and Paratyphi A. A separate branch of class IA, including serovar Heidelberg, Virchow, and Hadar, that had the highest number of FGCs, as well as serovars Montevideo, Schwarzengrund, Welterveden, Javiana, Kentucky and Tennessee, were commonly isolated in association with edible plants, constitute the class IE.

In addition of the 7 core FGCs, 5 highly conserved FGCs (*saf, ste, stf, sti* and *lpf*) were associated with *S. enterica* ssp. I (Tableau 3). The *sti*, *lpf* and *stf* clusters are missing in human specific serovars (class ID). The *ste* cluster is missing in class IA serovars and in some of the class IE serovars. Thus, *S. enterica* ssp. I harbours the core FGCs (*fim, bcf, sth, stb*, *stc-peg* and *std*), the conserved FGCs (*saf, ste, stf, sti* and *lpf*) and some sporadic FGCs unique to each serovar. Many FGCs of *Salmonella* are sporadic and form the unique repertoire in each serovars.

Despite the presence of many FGCs, extensive gene degradation was observed in most of the host-restricted and warm-blooded host adapted serovars, mainly Gallinarum, Choleraesuis, Paratyphi A and Typhi. Genome degradation of FGCs may correspond to the loss of genes rendered unnecessary by niche specialization or by selective pressure in order to diminish antigen presentation at the bacterial surface during systemic disease. Intriguingly, most of FGCs were intact in Paratyphi B.

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

Tableau 3. – Fimbrial distribution

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

1.4.1.5 Fimbrial regulation

Salmonella fimbriae are usually not expressed constitutively, and rarely expressed under laboratory condition, except for Fim fimbriae, a type-1 fimbria (54). Fimbriae are important during infection (70, 98, 99), suggesting that their expression is tightly regulated. Little is known about the regulation mechanisms that promote fimbrial expression. In general, fimbrial expression is positively or negatively regulated at the genetic level. Some regulators are unique to a specific fimbria, like the regulation of curli by CsgD, while others are global, like Dam, H-NS and Lrp (Leucine-responsive regulatory protein) (100). These mechanisms include regulatory proteins, DNA methylation, cyclic di-GMP, and small RNAs (100). In *Salmonella*, a regulation network exists between the virulence factors. Here, we present the regulation of fimbrial genes including the interaction with motility and invasion. Then, we propose an example of regulation of the *fim* FGC expression in *S.* Typhimurium, the most characterised fimbriae of *Salmonella*.

1.4.1.5.1 General regulation of fimbrial genes

Genes implicated in different aspects of virulence including motility, adhesion, invasion of host cells and intestinal persistence, are all regulated during infection. It was proposed that there is a temporal hierarchy between the T3SS of SPI-1 (invasion), flagellar and fimbrial genes, where SPI-1 is first activated, followed by flagellar genes and then type-1 fimbrial genes (*fim*). The crosstalk between these systems seems to be critical for bacterial pathogenesis (101). Each element of virulence is linked via a large regulatory network that is not completely understood. DNA adenine methylation (Dam) regulates many virulence genes in *Salmonella* (102): it is required for SPI-1 and *pef* expression but it also represses many genes, including the *std, csg* and flagellar genes (102-104). It was also shown that fimbrial FGCs are repressed by the Rcs phosphorelay, a sensor of outer membrane stress (105). Another example of regulation interaction between motility and fimbrial expression was observed by a deletion of *ydiV* in *S.* Typhimurium that results in the derepression of curli fimbriae (*csgAB*), causing an increase of swimming motility and a decrease of swarming (106).

Crosstalk regulation also occurs between the capsule and the type IVb fimbriae in *S.* Typhi. Both virulence factors are encoded on SPI-7 and facilitate invasion of monocytes, suggesting a regulation overlapped. However, the exact regulation elements that act on those two systems are unknown (107).

One of the posttranscriptional regulation mechanisms uses the binding of small RNAs and the Hfq chaperone. In an *hfq* mutant strain, the expression of fimbrial gene *sefA* was activated when most of the other fimbrial subunit genes were repressed in *S.* Enteritidis. Overall, the *hfq* deletion decreased adherence compared to wild-type strain. Thus, Hfq seems to regulate fimbrial expression of most fimbrial genes from *S.* Enteritidis (108). There is probably more sRNAs regulation of fimbrial gene expression awaiting to be discovered.

Phase variation is a transcriptional mechanism that controls the switch between fimbriated (ON) and afimbriated (OFF) cells within a bacterial population. In *Salmonella*, expression of *lpf* and *pef* was shown to be controlled by phase variation. The regulators of this mechanism are various and depend of the FGCs concerned (104, 109).

The secondary messenger cyclic-di-GMP controls virulence and biofilm formation in *Salmonella* (110). In *Salmonella*, curli expression was activated by AdrA, a GGDEF- domain protein that increases intracellular level of cyclic-di-GMP (111). Fimbrial production regulated by the cyclic-di-GMP level was also observed in other species such as *Klebsiella pneumonia*, *E. coli* and *P. aeruginosa* (112).

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

1.4.1.5.2 Regulation of fim in S. Typhimurium

The *fim* FGC codes for six genes (*fimAICDHF*). This cluster is the most studied and one of the most conserved fimbriae of *Salmonella enterica* and was mainly characterised in *S.* Typhimurium. These fimbriae have a binding specificity for mannose residues (112). The Fim fimbria of *Salmonella* is not homologous with its homonym from *E. coli*, except for sharing some morphological and mechanistic features (113, 114). Regulation and amino acid sequences of fimbrial proteins are divergent between the two species. The transition from afimbriated to fimbriated stage occurs for *fim*, but there is no inversion of the promoter region as observed for *E. coli* phase variation (115). For *S.* Typhimurium, the major subunit FimA is accompanied by two other subunits, FimI and FimF, and by the adhesin, FimH. The *fimC* and *fimD* genes encode respectively the chaperone and the usher (116). Ancillary genes, *fimZYW*, and a rare arginine transfer RNA (tRNA) *fimU* and *STM0551*, inserted between *fimY* and *fimW,* directly regulate *fim* expression (117).

Ancillary genes *fimZYW* regulate the expression of *fimA* (100, 118-120). FimZ, a sensor DNA-binding protein, is the principal positive regulator of *fimA* (121). FimY upregulates *fimZ* expression by binding to the *fimZ* promoter. FimY and FimZ then form a complex that activates the *fimA* promoter (112, 119). *fimY* is itself regulated by the arginine tRNA *fimU* (122). Lrp is another regulator that binds and activates the *fimZ* promoter, probably by antagonizing the binding of the global repressor protein H-NS to this promoter region (116, 120, 123). H-NS has a high affinity for AT-rich DNA region and *fimZ* gene has an unusual AT-rich sequence (123).

At the opposite, FimW repressed directly *fimA* expression and indirectly by lowering FimZ availability by its degradation caused by FimW binding (124). It is also suggested that STM0551, an –EAL domain protein, is a negative regulator of *fim* expression by lowering the c-di-GMP level (117).

FimZ is also used as a regulator relay by two-component systems for expression of *hilA*, the principal regulator of SPI-1 (invasion). The two-component system PhoBR induces *fimZ* expression and

PhoPQ activates *hilE*, one of the negative regulators of *hilA*, by a FimYZ-dependant manner. FimZ also down-regulates *flhDC*, genes implicated in flagella expression (125). Flagellar gene *fliZ* also represses *fimZ* on a posttranscriptional manner, reinforcing the fact that there is an alternated expression of flagella (motility) and fimbriae (adhesion) (101) and confirming a regulation network between SPI-1 (invasion) and fimbrial expression (126). Thus, a combination of factors directly implicated in fimbrial genes regulation can also impact on other virulence systems of *Salmonella*.

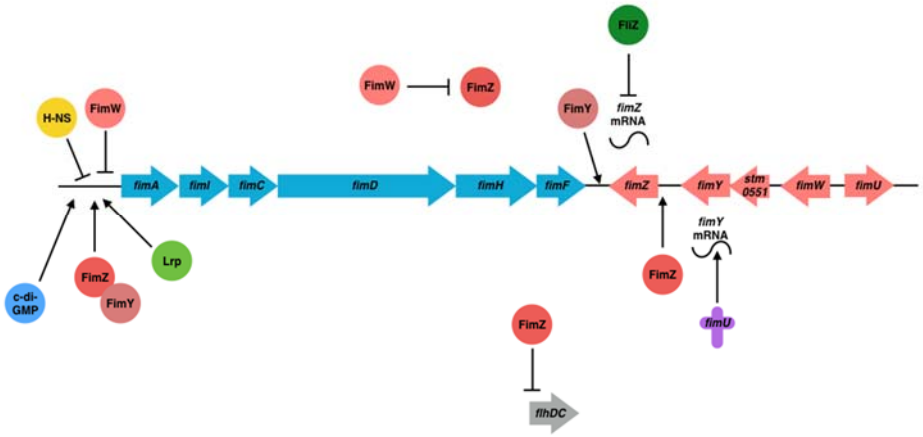

Figure 4. – *fim* regulation.

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

1.4.1.6 Fimbriae as a tool

Salmonella infections are a major concern for public and animal health. Some serovars are host-specific while others are broad-spectrum pathogens and can be transmitted from food-borne animals to humans. On the other hand, animals can develop health problems and will not be suitable for consumption. To prevent those issues, it is critical to develop ways to detect *Salmonella* and protect potential hosts against infection. The importance of fimbriae for detection of *Salmonella* by molecular techniques and for vaccines development is presented in this section (127).

1.4.1.6.1 Salmonella detection using fimbrial genes

Salmonella-specific tests were performed since the end of the 1980s and mainly targeted surface antigens. Those tests include agglutination tests and ELISA (enzyme-linked immunosorbent assays) (127-129). In 1993, Doran et *al.* presented a DNA-based test that targets *csgA* (*agfA),* offering a faster and more precise test for genus identification (130). Then, in early 2000s, PCR (polymerization chain reaction) tests using fimbrial genes, like *sef* or *csgA* (*agfA),* in combination with other virulence genes were developed to differentiate *Salmonella* strains from each other (131). Different PCR tests (multiplex, nested and direct PCR) were elaborated for detection of *Salmonella*. Several of those tests integrated detection of fimbrial genes (i.e. *staA*, *fimW*) to discriminate between serovars (131-133). Recently, a loop-mediated isothermal amplification (LAMP) assay was developed to detect *Salmonella* by targeting *bcfD,* a gene that belong to the core of FGC. In isothermal conditions, the reaction occurs in an hour permitting rapid detection of *Salmonella* (134).

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

1.4.1.6.2 Vaccine development

As surface structures, fimbriae constitute antigens of choice for the development of vaccines against *Salmonella* (51). Fimbriae are difficult to study because they are poorly expressed under laboratory conditions and are redundant. The most interesting fimbriae are the ones expressed during infection. Targeting those fimbriae will confer higher chances to be recognized by the immune system in key moments of infection.

More than twenty fimbrial antigens were detected in typhoid fever patient's blood by transcriptomic analysis: SteD, StaACD, BcfDE, SafBC, TcfBCD, StbBC, FimAIDH, StdBC, StgACD and SthA (135). Antibodies against immunogenic fimbrial proteins TcfB, StbD and CsgEFG were identified in the blood of typhoid fever patients (63). Immunoreactive antibodies against SthDA and BcfA were found in lymphocytes supernatant (ALS) of patients with typhoid fever (136).

SefA, a protein from the SEF14 fimbriae of *S.* Enteritidis, was used as an antigen associated with liposomes for oral immunisation of chickens (137). The immunisation of chickens by fimbrial antigens was efficient for IgG and IgA responses and reduced *Salmonella* colonisation. Four weeks after immunisation, the bacterial excretion from the intestinal tract was significantly reduced (137). The liposome-associated immunisation was also performed with fimbrial antigen from SEF21 and resulted in a similar efficiency (138). SefD, another antigen from SEF14, was also used to vaccinate animals in a bacterin preparation, a vaccine prepared from inactivated bacteria. This vaccine was efficient to reduce the presence of *Salmonella* from the spleens of hens (139).

As factors implicated in the first stages of infection, fimbriae are an interesting target for vaccine development (140). Fimbrial antigens are important for the development of new anti-*Salmonella* therapies (51, 135). However, a better understanding of their expression pattern *in vivo* is needed to optimize the therapeutic effects of fimbrial-targeted vaccines. Fimbrial antigens may be combined with other immunogenic proteins to increase the immune response (140).

1.4.1.7 Conclusion

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

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

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

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

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

1.5 Régulation génique

Le génome présente toute l'information nécessaire à la cellule bactérienne, mais doit être exprimé afin de répondre aux fonctions essentielles à la survie et à la virulence. Pour ce faire, les gènes d'intérêt sont liés aux promoteurs par une ARN polymérase (ARNpol) et ses cofacteurs pour être transcrits en ARN messager (ARNm). Cet ARNm est alors utilisé pour la traduction vers la protéine codée (141). Cette protéine peut à elle seule conférer un un effet visible (phénotype) à la bactérie ou être un élément d'un groupement plus grand menant à un phénotype. Plusieurs facteurs influencent l'expression d'un gène donné. Les microorganismes pathogènes utilisent une variété de signaux environnementaux pour guider l'expression de facteurs de virulence et l'établissement d'une infection efficace. Ces signaux peuvent être aussi variés que la concentration en solutés, la variation de pH, la variation thermique, la présence de certains nutriments, la source de carbone disponible et sa concentration, la présence d'oxygène ou la présence de différents perturbateurs (antimicrobiens, phages, sels biliaires, etc.) (142). Ces signaux sont alors perçus comme un stress par la bactérie qui doit y répondre pour survivre. La bactérie adaptée à réagir à ces environnements hostiles développe ainsi un arsenal lui permettant d'envahir cette niche et d'infecter de façon optimale son hôte. Dans cette section, nous verrons les régulateurs influençant la transcription des gènes d'intérêt et la gestion des signaux environnementaux.

1.5.1 Facteurs sigma

Parmi les régulateurs principaux de la transcription, sont retrouvés les facteurs sigma (σ) qui sont des sous-unités de l'ARNpol et agissent directement sur la section promotrice d'un gène en union avec l'ARNpol. En fait, chaque facteur σ agit en liant l'ARNpol, puis la guide vers ses gènes cibles spécifiques. Il est important de mentionner que chaque bactérie possède une seule ARNpol et que les facteurs σ pouvant s'y associer sont la clé de la spécificité de transcription et agissent en conditions précises (143). RpoD ou σ^{70} est le facteur principal pour activer les gènes de ménage (''*housekeeping genes*''). RpoS ou σ^{38} est le principal facteur impliqué dans la majorité des situations de stress chez la bactérie. RpoE ou

σ^{24} est impliqué dans le stress extracytoplasmique et aux températures extrêmes. RpoH ou σ^{32} est impliqué dans la régulation des stress cytoplasmiques et des chocs de température. RpoN ou σ^{54} est le facteur important en cas de limitation en azote ou un de ses composés. RpoF ou σ^{28} serait nécessaire à la formation de flagelles par la bactérie.

Les facteurs σ peuvent être eux-mêmes inhiber dans certaines situations par des anti-facteurs σ. Par exemple, DnaK inhiberait σ^{32}, tandis que certaines protéines améliorant la liaison (''*enhanced binding proteins*'' ou EBP) inhiberaient σ^{54} (144).

1.5.2 Régulateurs liant l'ADN

Les facteurs de transcription sont classés en plusieurs familles selon au moins deux domaines qui permettent leur action. Le premier domaine joue habituellement un rôle de senseur de signal envers un ligand, tandis que le deuxième domaine joue essentiellement le rôle clé de régulation en liant l'ADN cible. Ces deux domaines peuvent être présents sur une même protéine régulatrice ou en un système régulateur comme les systèmes à deux composantes (TCS ou ''*Two-component system*''). Chez les bactéries, le motif régulateur le plus commun est hélice-tour-hélice (145).

1.5.2.1 Régulateurs transcriptionnels de type LysR (LTTR)

Les LTTR sont très communs chez les procaryotes. Ils sont constitués de 300 à 350 acides aminés et leurs structures protéiques sont très similaires. Ce type de régulateur active la transcription de gènes et d'opérons aux fonctions variées incluant la virulence, la gestion du stress oxydatif, l'utilisation d'acides aminés et la fixation de l'azote (146). Chez les LTTR, le domaine liant l'ADN cible possède un motif hélice-tour-hélice et la plupart de ces facteurs nécessite une petite molécule liant leur section C-terminale et servant ainsi de co-inducteur (147). Chez *S*. Typhimurium, 44 LTTR sont dénombrés, incluant OxyR, LeuO, LysR, HdfR et MetR (148). Chez *S.* Typhi CT18, le site Uniprot dénombre 45 LTTR.

1.5.2.2 Facteurs de transcription de la famille CAP

Les facteurs de transcription de la famille CAP (''*Catabolite activator protein*'') possède aussi un motif hélice-tour-hélice pour lier l'ADN. Par contre, leur extrémité senseur nécessite la liaison d'une molécule effectrice pour changer la conformation du facteur de l'état inactif à l'état actif. Les régulateurs présents chez *S.* Typhi de cette famille sont Crp (CAP) et FNR. Crp est impliqué dans la répression catabolique, FNR dans la réduction du nitrate et du fumarate en absence d'oxygène (149).

1.5.2.3 Autres types de régulateurs liant l'ADN

Les facteurs de transcription sont classés en d'innombrables familles selon leurs homologies de séquence. Deux familles de régulateurs ont été introduits ci-haut, mais plusieurs autres participent à capter et réagir aux différentes conditions environnementales. Parmi ces familles, il y a les EBP qui agissent en activant ou inhibant σ^{54} en réponse à l'azote (150). De plus, SlyA, un membre de la famille MarR, est impliqué dans la virulence et la survie dans les macrophages chez *S.* Typhimurium (151). Plusieurs autres exemples de régulateurs liant l'ADN pourraient être cités chez *S.* Typhi, dont les régulateurs de la famille Fur, RfaH, ArgR, SeqA, PfkB et plusieurs autres (Uniprot).

1.5.3 Systèmes à deux composantes

Les bactéries ont développé des systèmes permettant d'évaluer leur environnement grâce à un senseur, puis de répondre aux contraintes environnementales ressenties grâce à un régulateur. *S.* Typhi possède 30 de ces systèmes à deux composantes (TCS) ressentant et contrôlant l'environnement bactérien (152). Le senseur du TCS possède normalement deux domaines : un domaine qui perçoit le milieu, tandis que le deuxième domaine, qui possède une activité kinase, et transmet le signal reçu par phosphorylation d'un résidu histidine. Le régulateur possède aussi deux domaines, c'est-à-dire un domaine receveur avec un résidu aspartate et un domaine réponse. Typiquement, le senseur est inséré dans la membrane interne de la bactérie et s'autophosphoryle suite à la stimulation reçue par l'environnement. Cette autophosphorylation est dépendante de l'ATP. Le senseur phosphorylé transmet alors son groupement phosphate au domaine receveur du régulateur. Le régulateur ainsi

phosphorylé est activé et peut lier ses régions cibles de l'ADN. Le régulateur du TCS peut activer ou réprimer des gènes cibles de façon à modifier le comportement bactérien en fonction de l'environnement rencontré (153).

1.6 Réponse à l'environnement

Les différents facteurs de transcription répondent à un besoin de la bactérie suite à un signal perçu par celle-ci. Différents signaux environnementaux sont possibles et ces signaux engendrent des réponses variées. Dans cette section, un résumé des principaux stress retrouvés par *Salmonella* et de la réponse bactérienne à ces situations est présenté.

1.6.1 Déstabilisation des membranes bactériennes

L'enveloppe bactérienne de *Salmonella* est de type Gram négative, c'est-à-dire qu'elle possède une membrane interne, un espace périplasmique incluant une couche de peptidoglycanes et une membrane externe. Une régulation rigoureuse de ces composantes doit être maintenue en tout temps afin de conserver la forme de la cellule et de protéger la cellule contre l'extérieur. Les membranes sont composées de plusieurs phospholipides possédant une tête hydrophile et une queue hydrophobe : les queues hydrophobes se rassemblent au centre de la membrane, protégées par les têtes hydrophiles. Plusieurs protéines sont imbriquées dans chacune des deux membranes afin de permettre le passage de différents éléments (ions, protéines, autres nutriments). La membrane externe est aussi composée des LPS sur son feuillet externe (154). La couche de peptidoglycanes retrouvée dans le périplasme est la clé de la forme de la bactérie et aussi une défense importante contre les conditions environnementales changeantes. Plusieurs éléments stressants peuvent déstabiliser la membrane, dont le changement de température, les changements osmotiques et les changements d'acidité. Une température plus chaude déstabilise les membranes en augmentant la fluidité de ses composantes. Les changements osmotiques peuvent être causés par un débalancement de la concentration de solutés dans l'environnement : une augmentation de la salinité externe entraîne une sortie d'eau de la cellule

bactérienne, tandis qu'une diminution de la salinité augmentera l'entrée d'eau. Certains sels, comme les sels biliaires, ont aussi une activité antimicrobienne causant une déstabilisation de la membrane. L'acidité environnementale peut être définie comme la présence d'ions H+. Le potentiel membranaire dépend de la concentration d'ions présente de chacun des côtés de la cellule bactérienne, c'est-à-dire entre le cytoplasme bactérien et l'espace extracellulaire. Le stress membranaire est régulé par la bactérie à l'aide de plusieurs régulateurs. Premièrement, le TCS OmpR/EnvZ capte et régule autant la réponse à une haute salinité que l'augmentation de pH (155). D'autres TCS sont impliqués dans la réponse au stress osmotique ou la réponse à la salinité, soit PhoPQ, RcsBC, CpxAR, BaeRS et SirA/BarA (156-160).

1.6.2 Limitation en nutriments essentiels

La présence de nutriments en quantité suffisante est essentielle à la survie bactérienne. Pour le maintien de ses fonctions, la bactérie a besoin de source de carbone, d'azote, d'hydrogène, de phosphate, de potassium, de souffre et de plusieurs éléments traces comme le sodium, le calcium, le fer, le zinc, le cuivre et le manganèse (161). La bactérie a donc développé plusieurs mécanismes pour reconnaître et réagir à la limitation de ces nutriments les plus importants pour *Salmonella*.

Le carbone est un des éléments les plus importants pour tout organisme vivant, car il est central autant à la formation d'acides aminés et de protéines, que dans la formation des acides nucléiques formant l'ADN. En bref, le carbone est l'élément principal des structures du vivant. Le carbone est aussi retrouvé dans les sucres que la bactérie capte et utilise pour la formation de son énergie. La source préférentielle de carbone est le glucose, un monosaccharide. Par la suite, les sucres sont classés, pour chaque espèce bactérienne, dans un ordre d'utilisation selon la préférence métabolique. Le principal régulateur régulant les gènes responsables du transport et de l'utilisation des différents sucres est nommé Crp (''*cAMP receptor protein*'') ou anciennement CAP (''*Catabolite activator protein*''). La protéine Crp est activée en présence d'adénosine monophosphate cyclique (cAMP). Le cAMP s'acolle à la région senseur de Crp et le facteur peut alors lier l'ADN par son extrémité liant l'ADN (162). En présence de glucose,

l'enzyme IIA^{Glc} ($EIIA^{Glc}$) du système phosphoénolpyruvate (PEP) est phosphorylé et inhibe l'activité de l'adénylate cyclase nommée CyaA. CyaA est responsable de la formation de cAMP. La production de cAMP est donc inhibée en présence de glucose, ce qui empêche l'activation du régulateur Crp, empêchant ainsi l'activation du transport ou du métabolisme des sucres secondaires comme le lactose, le mannose, le fucose, le fructose ou autres (163). D'autres régulateurs dont Cra (FruR), FNR, AraC régissent de façon secondaire l'utilisation de sources de carbone en conditions variées (164). Plusieurs de ces régulateurs ont un rôle établi pour la virulence de plusieurs espèces bactériennes. Par exemple, Crp régulerait le fimbria Pap chez *E. coli* uropathogénique (165).

L'azote est aussi un élément clé de la formation des acides aminés et acides nucléiques. Il peut aussi devenir un élément important de la chaîne respiratoire en absence d'oxygène et ainsi produire des espèces réactives néfastes à la bactérie. Le facteur σ^{54} est à la base de la régulation de l'azote, que ce soit son transport à l'intérieur de la cellule comme son utilisation pour former les molécules essentielles. Un TCS, GlnGH, aussi appelé NtrCB, capte les différences de concentration en ammonium ($NH4^{+}$) et agit sur σ^{54} en plus de plusieurs autres EBP (166, 167). La réponse stringente est la principale réponse impliquée dans la limitation en acides aminés et agit aussi sur σ^{54} en situation de stress. Cette réponse est médiée par RelA et SpoT principalement qui permettent la production de ppGpp, aussi nommé alarmone. L'accumulation de ppGpp active alors σ^{54}, ce qui augmente la captation d'azote de l'environnement, principalement sous forme d'ammonium (34). Comme présenté ci-haut, la gestion de l'azote nécessite un réseau complexe de régulation pour permettre en finalité la formation d'acides aminés et d'acides nucléiques. Ce réseau régulatoire mène aussi à l'activation de gènes de virulence chez plusieurs bactéries (168, 169). Chez *S.* Typhimurium, DskA, une petite protéine liant le ppGpp serait importante dans la régulation de la motilité, de la formation de biofilm et la colonisation de l'intestin (169).

Le fer est un métal essentiel à la grande majorité des êtres vivants. Il est aussi très important à la chaine respiratoire bactérienne et est retrouvé de façon relativement ubiquitaire dans l'environnement. *S.*

Typhi utilise des systèmes d'acquisition et de transport spécialisés pour s'assurer d'une concentration optimale de fer dans sa cellule. Premièrement, des sidérophores, c'est-à-dire des chélateurs biologiques de fer, sont synthétisés. Deux types de sidérophores sont retrouvés chez *S.* Typhi, soit les entérobactines et les salmochellines. *S.* Typhi produit une plus grande quantité de sidérophores que *S.* Typhimurium et principalement des entérobactines (170). Ces sidérophores sont exportés hors de la cellule bactérienne par EntS et IroC au niveau de la membrane interne, puis par TolC à la membrane externe. Les sidérophores s'associent alors au Fe^{3+} et l'importe dans la bactérie par des transporteurs de type ABC (''*ATP binding cassette*''). Le fer est alors relâché par hydrolyse des sidérophores. Le Fe^{2+} peut librement passer les membranes via des porines et les systèmes Feo, Sit et MntH. L'homéostasie du fer chez *Salmonella* est régulée principalement par Fur. Notre laboratoire a précédemment démontré l'importance de Fur sur la virulence de *S.* Typhi (170, 171).

1.6.3 Respiration aérobie et espèces réactives à l'oxygène

En condition aérobique, l'oxygène utilisé lors de la respiration est transformé en peroxyde (H_2O_2), en radicaux hydroxyles (HO•) et en superoxyde (O_2-) principalement. Ces trois molécules sont nommées espèces réactives à l'oxygène ou espèces oxydatives. Trois régulateurs sont au centre de la détoxification des espèces réactives, c'est-à-dire OxyR et SoxRS. Les espèces réactives peuvent être détoxifiées par l'activité de catalases et de peroxydases. *Salmonella* possède trois calases (KatE, KatG et KatN) et trois peroxydases (AhpC, Tpx et TsaA) (172).

La respiration permet principalement la production d'énergie sous la forme d'adénosine triphosphate (ATP). Ce processus se produit par échange d'électrons de donneurs vers accepteurs par réaction rédox en une chaine complexe d'événements. Le transfert d'électron est couplé à un mouvement de protons vers l'intérieur de la bactérie, ce qui cause un gradient permettant la création de l'ATP. Chez les bactéries, plusieurs donneurs et accepteurs d'électron sont possibles. Les électrons peuvent entrer par trois moyens dans la chaine de transport d'électron à partir de leur donneur, soit par des déshydrogénases, par l'ensemble des quinones (ubiquinones, ménaquinones) ou par le cytochrome.

Certaines de ces entrées des électrons sont aussi des pompes à protons qui transfert un proton du cytoplasme vers le périplasme en réponse au mouvement d'électron : les pompes à protons sont la base de la chaine de transport d'électron et permettent le gradient favorisant l'activité de la synthase d'ATP. L'accepteur final d'électron est l'oxygène dans le cas où il est disponible à la bactérie (173). Par contre, d'autres molécules peuvent remplacer l'oxygène tel que le tétrathionate largement étudié chez *S.* Typhimurium. La capacité d'utiliser le tétrathionate plutôt que l'oxygène pour sa respiration confère à la bactérie pathogène un avantage pour la colonisation de l'intestin (174).

1.7 Hypothèse, but et objectifs

S. Typhi code 14 systèmes fimbriaires dont 12 de type chaperon-placier. Ceux-ci sont présents en une combinaison unique associée à ce sérovar. En fait, certains de ces fimbriae sont aussi présents chez *S*. Typhimurium, par exemple, mais certains sont uniques à *S*. Typhi et la combinaison finale est caractéristique à ce sérovar. Ces 12 systèmes d'adhésion sont peu exprimés et peu connus chez *S.* Typhi, ce qui fait que leur rôle et leur régulation reste à découvrir. Parmi ceux-ci, deux fimbriae ont été amplement étudiés chez *S.* Typhimurium, c'est-à-dire Fim et Std.

Nous croyons que nous pourrons identifier de nouveaux rôles pour les fimbriae de type chaperon-placier chez *S.* Typhi. Nous pensons aussi identifier de nouveaux facteurs régulant certains de ces fimbriae et ainsi identifier des mécanismes bactériens reliés à la reconnaissance de stress environnementaux permettant l'expression des fimbriae chez *S.* Typhi. Lorsque *S*. Typhi parcourt le corps humain tout au long de sa pathogenèse, il rencontre différents signaux environnementaux qui devraient activer ou inhiber différents fimbriae.

Le but du projet actuel est d'analyser fonctionnellement les fimbriae de type chaperon-placier de *S.* Typhi. Pour ce faire, nous avons caractérisé chacun des douze fimbriae de façon générale pour prédire leur rôle sur la pathogenèse de *S.* Typhi. Ensuite, nous nous sommes concentrés sur l'étude de la

régulation de *std* et *fim* afin de déterminer de nouveaux facteurs influençant ces deux opérons fimbriaires. Par l'identification de nouveaux régulateurs de *std* et *fim*, nous avons pu mettre en lumière deux mécanismes importants à la production de fimbriae chez *S.* Typhi. Ces mécanismes sont impliqués dans la gestion de stress environnementaux que la bactérie peut rencontrer durant son cycle d'infection.

Chapitre 2 – Caractérisation des fimbriae de type chaperon-placier chez *Salmonella enterica* sérovar Typhi

2.1 Préface au chapitre

Une caractérisation généralisée des 12 fimbriae de type chaperon-placier de *S.* Typhi a été réalisée afin de déterminer l'expression de leur promoteur en différentes conditions mimant l'infection, de confirmer leur assemblage à l'extérieur de la bactérie, ainsi que de déterminer l'effet de chacun de ces fimbriae sur la pathogenèse de *S.* Typhi. Cette première étude des fimbriae de *S.* Typhi a permis de mettre en lumière les fimbriae aux effets les plus intéressants. Cet article a été révisé par un comité de pairs et accepté à la revue à accès ouvert «*Frontiers in Cellular and Infection Microbiology*».

Article 2 : Karine Dufresne, Julie Saulnier-Bellemare and France Daigle. Functional analysis of the chaperone-usher fimbrial gene clusters of *Salmonella enterica* serovar Typhi. *Front Cell Infect Microbiol*. 2018; 8: 26. doi: 10.3389/fcimb.2018.00026

Contribution des auteurs :

Le projet a été élaboré par France Daigle. J'ai réalisé les expérimentations, j'ai produit les tableaux et graphiques ainsi et j'ai compilé et analysé les diverses données. La souche afimbriaire a été réalisée par Élise David, puis plusieurs des vecteurs de l'étude ont été construits par Yoan Houde. Julie Saulnier-Bellemare a réalisé les expériences de production de biofilms en plaque. J'ai écrit le manuscrit sous supervision de France Daigle et tous les co-auteurs ont révisé celui-ci. Les fonds nécessaires au projet ont été fournis par France Daigle.

2.2 Article 2: Functional Analysis of the Chaperone-Usher Fimbrial Gene Clusters of *Salmonella enterica* serovar Typhi

Karine Dufresne, Julie Saulnier-Bellemare and France Daigle

Keywords : chaperone-usher, fimbriae, *S.* Typhi, infection, biofilm, pathogenesis

2.2.1 Abstract

The human-specific pathogen *Salmonella enterica* serovar Typhi causes typhoid, a major public health issue in developing countries. Several aspects of its pathogenesis are still poorly understood. *S.* Typhi possesses 14 fimbrial gene clusters including 12 chaperone-usher fimbriae (*stg*, *sth*, *bcf*, *fim*, *saf*, *sef*, *sta*, *stb*, *stc*, *std*, *ste,* and *tcf*). These fimbriae are weakly expressed in laboratory conditions and only a few are actually characterized. In this study, expression of all *S.* Typhi chaperone-usher fimbriae and their potential roles in pathogenesis such as interaction with host cells, motility, or biofilm formation were assessed. All *S.* Typhi fimbriae were better expressed in minimal broth. Each system was overexpressed and only the fimbrial gene clusters without pseudogenes demonstrated a putative major subunits of ~ 17 kDa via SDS-PAGE. Six of these (Fim, Saf, Sta, Stb, Std, and Tcf) also show extracellular structure by electron microscopy. The impact of fimbrial deletion in a wild-type strain or addition of each individual fimbrial system to an *S.* Typhi afimbrial strain were tested for interactions with host cells, biofilm formation and motility. Several fimbriae modified bacterial interactions with human cells (THP-1 and INT-407) and biofilm formation. However, only Fim fimbriae had a deleterious effect on motility when overexpressed. Overall, chaperone-usher fimbriae seem to be an important part of the balance between the different steps (motility, adhesion, host invasion and persistence) of *S.* Typhi pathogenesis.

2.2.2 Introduction

Salmonella enterica serovar Typhi is a human-specific pathogen responsible for a systemic disease called typhoid fever. It causes ~22 million infections and 200,000 deaths annually worldwide (WHO, 2012; Qamar et al., 2015). Over the years, the number of cases has increased, but the use of antibiotics has controlled the eventual burden. However, the increased emergence of multidrug resistant *S.* Typhi strains can complicate treatment and can lead to a higher death rate (Rowe et al., 1997; Thong et al., 2000; Pokharel et al., 2006; WHO, 2012). A better understanding of *S.* Typhi pathogenesis is required to better control and treat typhoid (Obaro et al., 2017).

Salmonella species enter their host by the intestinal tract and cross the intestinal barrier (Clark et al., 1994). *S.* Typhi invades the human host by a variety of virulence factors such as two type III secretion systems (T3SS) encoded by *Salmonella* pathogenicity islands (SPI)−1 and−2, the presence of 18 SPIs in the genome, the human-restricted typhoid toxin and the flagella (Galan and Zhou, 2000; Galán, 2001; Waterman and Holden, 2003; Chang et al., 2016; Horstmann et al., 2017). It also evades the host innate immune response by the production of an extracellular capsule, the Vi antigen, encoded on SPI-7 (Wilson et al., 2008; Winter et al., 2008; Wangdi et al., 2014). Most of the data available about *S.* Typhi pathogenesis and virulence factors is based on the systemic infection of mice with *S.* Typhimurium. Due to this lack of direct information, crucial questions still remain concerning host-specificity and pathogenicity mechanisms of *S.* Typhi.

Fimbriae are proteinaceous extracellular structures mainly involved in adhesion, a crucial initial step for colonization and entry into host cells. Fimbriae have also been shown to contribute to interactions with macrophages, intestinal persistence, biofilm formation and bacterial aggregation in other *Salmonella* serovars (Edwards et al., 2000; Zhang et al., 2000; Boddicker et al., 2002; Tsui et al., 2003; Weening et al., 2005; Ledeboer et al., 2006). Fimbriae are grouped into three classes according to their mode of assembly. The curli fimbriae are assembled by a process called nucleation-precipitation where the major subunits are precipitated together by the presence of the nucleator in the extracellular medium. The type IV fimbriae are assembled at the

inner-membrane platform and extended through the periplasm and outer membrane to the extracellular environment. This fimbria can be assembled or disassembled using ATP. Lastly, the chaperone-usher (CU) fimbriae use a periplasmic chaperone and an outer-membrane usher to assemble the major subunits into the final external filamentous structures. This class of fimbriae is the most diverse and *S.* Typhi fimbriae are phylogenetically sub- classified into five clades based on the usher: γ_1 , γ_3 , γ_4 , π, and α (Figure 5; Townsend et al., 2001; Nuccio and Bäumler, 2007).

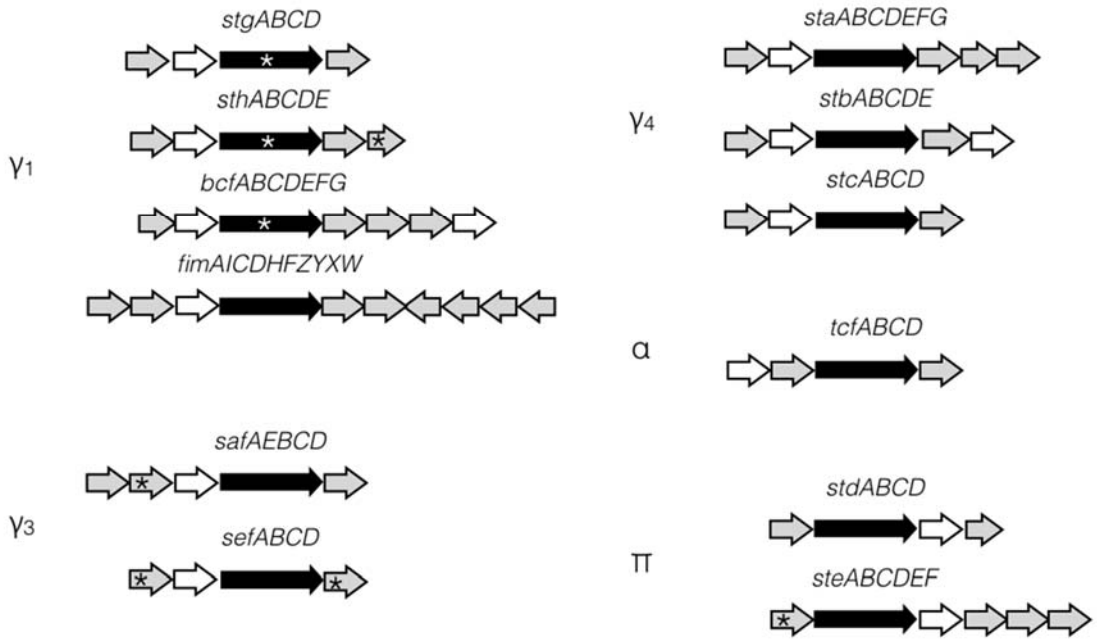

Figure 5. – *S.* Typhi CU fimbrial operons organization.

S. Typhi possesses 12 putative fimbrial gene clusters divided in 5 clades (γ_1, γ_3, γ_4, α and π) depending of the usher homologies. Stg, Sth, Bcf and Fim are γ_1 fimbriae, while Saf and Sef are in clade γ_3 and Sta, Stb and Stc are in clade γ_4. Tcf is the only representative of α clade. Std and Ste are π fimbriae. Ushers are represented by black arrow and chaperones by white arrow. No distinction is made for major / minor subunits, adhesins or other proteins (grey arrows). Pseudogenes are marked by asterisk.

The *S.* Typhi genome possesses a unique repertoire of 14 putative fimbrial clusters identified by whole-genome sequencing (Humphries et al., 2003) including 12 CU fimbriae (Townsend et al., 2001). However, only 3 CU fimbriae (Stg, Sta, and Tcf) were previously studied (Forest et al., 2007; Bishop et al., 2008; Berrocal et al., 2015; Leclerc et al., 2016; Gonzales et al., 2017), mainly due to the weak expression of most of these fimbriae under laboratory conditions (Low et al., 2006; De Masi et al., 2017). Among the CU fimbriae, 5 (*stg*, *sef*, *sta*, *ste,* and *tcf*) are present in *S.* Typhi but absent in the well-studied broad-range pathogen *S.* Typhimurium and 5 of the fimbrial gene clusters in *S.* Typhi (*stg*, *sth*, *bcf*, *sef*, and *ste*) have at least one pseudogene. Approximately 5% of the *S.* Typhi genome contains pseudogenes, which were often associated with its host-specificity and may restrict *S.* Typhi only to the human host (Baker and Dougan, 2007). In fimbrial putative gene clusters, pseudogenes are present in the usher genes (*stgC*, *sthC,* and *bcfC*), but also in subunits or adhesin genes (*sthE*, *sefA*, *sefD,* and *steA*). However, *S.* Typhi mutants with a deletion of *stg* demonstrated decreased infection of cell lines, suggesting a potential function for this fimbrial cluster despite the presence of a pseudogene in the usher gene (Forest et al., 2007; Berrocal et al., 2015; Gonzales et al., 2017). Allelic variation, especially for the FimH adhesin of the Type I fimbria, was studied in several serovars and may also be implicated in host tropism for *Salmonella* (Kisiela et al., 2012; Yue et al., 2015; De Masi et al., 2017).

Here, we hypothesize that some of 12 CU fimbriae of *S.* Typhi are produced and involved at different steps of pathogenesis despite their poor fimbrial expression and presence of pseudogenes. The characterization of all 12 CU fimbriae of *S.* Typhi includes expression levels under the tested conditions, surface structure assembly, interactions with host cells, role in biofilm production and in motility. Overall, each of the S. Typhi fimbrial systems were found to contribute to different steps of the pathogenesis process and six of these produced visible fimbriae.

2.2.3 Material and methods

2.2.3.1 Bacterial strains, plasmids and growth conditions

The list of bacterial strains and vectors used in this study is given in Tableau 5. Bacteria were routinely grown overnight at 37°C on Luria-Bertani (LB) agar plates or with agitation in LB broth. When required, supplements or antibiotics were added at the following concentrations: 0.05–1mM IPTG, 50μg/ml diaminopimelic acid (DAP), 50 μg/ml kanamycin, 50 μg/ml ampicillin, and 34μg/ml chloramphenicol. IPTG is used to induce expression of fimbrial cluster cloned into pMMB307c (Morales et al., 1991) and DAP is an amino acid that allows the maintain of the conjugative strain MGN-617 containing an *asd* mutation (Kaniga et al., 1991). Transformation of bacteria was performed by using the calcium/manganese based (CCMB) or electroporation methods as previously described (O'Callaghan and Charbit, 1990).

2.2.3.2 Cloning of fimbrial promoters and β-galactosidase assays

The primers used for cloning of fimbrial promoters are listed in Tableau 6. The promoter region upstream of each gene cluster was predicted by the Softberry software BPROM (www.softberry.com) and amplified by PCR reaction. PCR fragments between 170 and 730 bp were cloned upstream of the promoterless *lacZ* gene in vector pRS415. The resulting vector was transformed into *S.* Typhi WT strain. Expression of each promoter was measured by β-galactosidase assays following growth under different culture conditions. LB was used as a classic rich laboratory medium and bacteria were inoculated in broth or on agar and incubated overnight at 37°C. M63 was used as a minimal medium and was prepared as previously described (Leclerc et al, 2013). Bacteria were inoculated in M63 broth or on M63 agar. For the induction of the T3SS encoded on SPI-1, the bacteria were grown in LB 0,3M NaCl, and incubated overnight at 37°C without agitation for low oxygenation (Lee et al., 1992; Weinstein et al., 1998). For the induction of T3SS encoded on SPI-2, the bacteria were incubated in LPM broth, pH 5.8, and incubated overnight at 37°C with agitation (Coombes et al., 2004). For each condition, β-galactosidase activity was assessed using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as described previously (Miller, 1972).

2.2.3.3 Chromosomal deletion of fimbrial gene clusters

The primers used for mutagenesis are listed in Tableau 6. Mutant strains for each fimbrial gene cluster were obtained by allelic exchange mutagenesis as previously described (Forest et al.,2007) except for the deletion of *sta*, that was obtained by λ red recombination system (Datsenko and Wanner, 2000). Each fimbrial deletion was verified by PCR (data not shown). The afimbrial strain resulted from the successive deletion of each of the fimbrial clusters, including the deletion of genes encoding the curli (*csg*) and the type IV fimbriae (*pil*).

2.2.3.4 Cloning of the fimbrial gene clusters

The primers used for cloning of fimbrial gene clusters are listed in Tableau 6. Fimbrial gene clusters, with or without the promoter region, were amplified by PCR using Q5 High-Fidelity DNA polymerase (New England Biolabs). Fragments between 4.5 and 9.7 Kb were then cloned in IPTG-inducible vector pMMB207c (fimbrial gene cluster without promoter region) (Morales et al., 1991) or into the low-copy vector pWSK29 (fimbrial gene cluster with its native promoter region) (Wang and Kushner, 1991). These constructions were transformed by electroporation into the afimbrial *S.* Typhi strain.

2.2.3.5 SDS-PAGE and transmission electron microscopy

S. Typhi containing the inducible vector pMMB207c with or without each fimbrial gene cluster was grown overnight at 37°C LB plates with chloramphenicol and 50 µM IPTG. Bacteria were harvested in LB broth. For SDS-PAGE, bacteria were washed with a solution of 0.9% sodium chloride and then with a solution of 75mM sodium chloride and 0.5 mM Tris pH 7,4. Extracellular proteins were extracted by heat treatment at 60°C during 15 minutes and were precipitated by addition of 10% trichloroacetic acid (Beloin et al., 2006). The concentration of proteins was normalized. The samples were loaded on SDS-PAGE 15% and stained with Coomassie R-250. The band of interest for Std and Stc (StdA and StcA) were cut from the gel, destained and digested by

trypsin. Peptides were sequenced using LC-MS/MS at the Center for Advanced Proteomics Analyses (IRIC, Université de Montréal).

For electron microscopy, the 3-24 hours-induced cultures were fixed with 2% glutaraldehyde for 30 minutes and adsorbed onto nickel formvar-carbon coated grids for 10 minutes and stained with 1% phosphotungstic acid. Bacteria were observed under Philips CM-100 or Hitachi H-7100 electron microscope.

2.2.3.6 Interactions with human epithelial intestinal cells

INT-407 (Henle) cells (ATCC CCL-6) were grown in minimal essential medium supplemented (Wisent) with 10% heat-inactivated fetal bovine serum (FBS) (Wisent) and 25 mM HEPES (Wisent). The assays were then performed as previously described with MOI of 20 (Forest et al., 2007).

2.2.3.7 Interactions with macrophages

THP-1 cells (ATCC TIB-202) were maintained in RPMI 1640 (Wisent) supplemented with 10% heat-inactivated FBS (Wisent), 1 mM sodium pyruvate (Wisent) and 1% MEM non-essential amino acids (Wisent). The assays were then performed as previously described at a MOI of 10 (Daigle et al., 2001).

2.2.3.8 Biofilm assays

The protocol was adapted from Ganjali Dashti et al. (2016). Bacteria were grown overnight in LB broth. The cultures were then diluted 1:10 in nutrient broth containing bile, glucose and potassium and incubated 72 hours statically at 37°C. The biomass production was determined by the crystal violet assay as described by Tremblay et al. (2015). The assay was carried out at least three times for each bacterial strain.

2.2.3.9 Motility assays

The motility assays were as previously described (Sabbagh et al., 2012). Bacteria were grown in LB broth overnight with agitation at 37°C and diluted 1/100 prior to puncture of the agar plate for mutant strains and afimbrial strains with pWSK29 vector. For afimbrial strains with inducible vector pMMB207c, the overnight cultures were diluted 1/100 in new medium and the strains were induced with 1mM IPTG for 3 hours at OD_{600} value of 0,6 and then punctured in the agar plate. Bacterial strains were accompanied by the control strain on each agar plate for comparison. Plates were incubated for 16 hours at 37°C. The diameter (mm) was measured and each construction was tested at least in triplicates. The results are presented as the mean ratio of the tested strain/wild-type ± SEM of the replicas.

2.2.3.10 Statistical Analysis

Statistical analysis was performed on GraphPad Prism. Two-tailed unpaired Student's *t*-test was applied on data sets for each construction compared to their control data. $P < 0.001$ was considered extremely significant (***); $P < 0.01$ was considered very significant (**) and $P < 0.05$ was considered significant (*).

2.2.4 Results

2.2.4.1 *S.* Typhi CU fimbriae are better expressed in minimal media

The first step to characterize the CU fimbriae of *S.* Typhi was to determine the best *in vitro* condition of expression of each fimbria. As most fimbriae are often poorly expressed during growth under classic laboratory conditions, each fimbrial promoter was cloned in fusion with the reporter gene *lacZ* on a multicopy vector (pRS415). The β-galactosidase activities of these transcriptional fusions were then assessed in 6 different conditions including conditions that induce the *Salmonella* T3SSs and that may mimic possible environmental cues encountered by *S.* Typhi. At first glance, the expression pattern seems similar regardless of the tested condition with the highest expression for the *saf* promoter followed by *std*, *sth* and *stc*, whereas *sta*, *stb* and *fim*

had the lowest expression (Figure 6). All fimbrial promoters showed their highest expression in minimal medium and the lowest when SPI-1 was induced, except for *stc* and *std*. altogether, promoters reacted to different growth conditions and demonstrated variation in their expression.

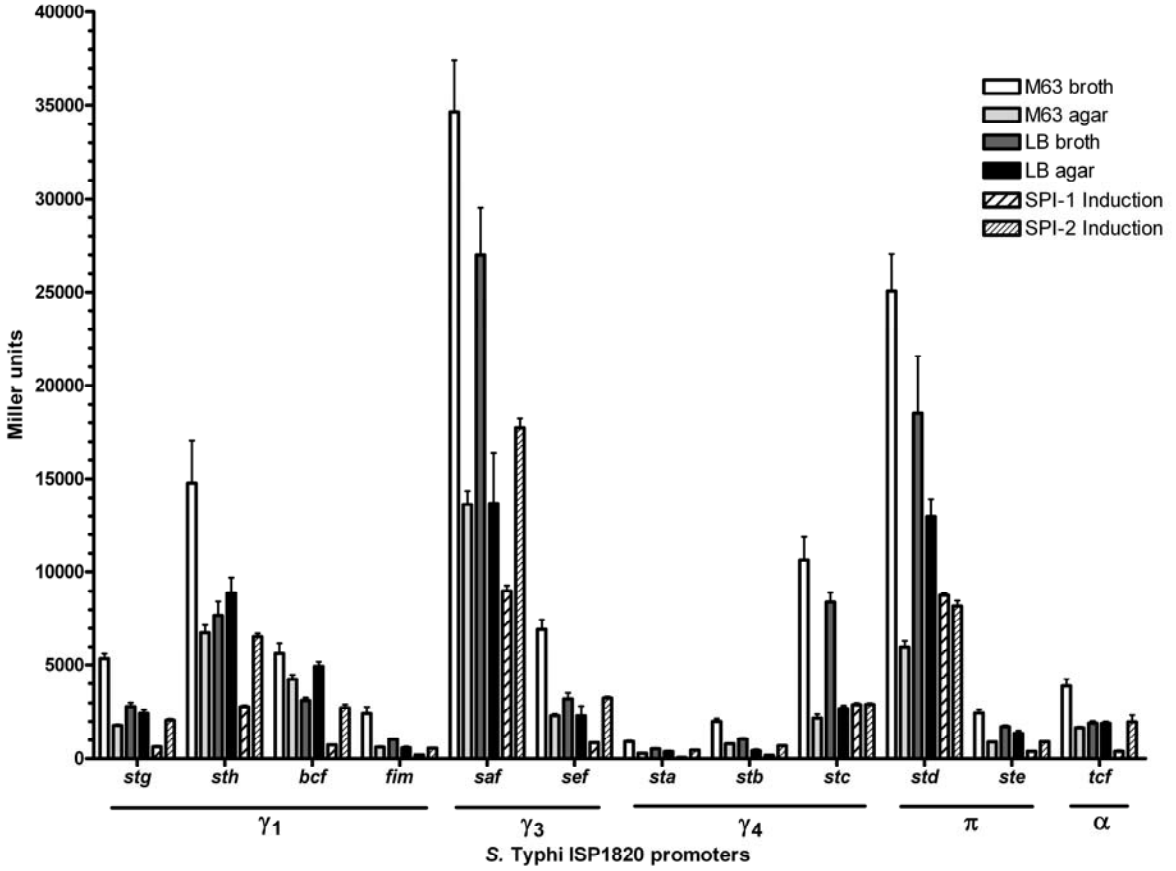

Figure 6. – Expression of *S.* Typhi ISP1820 CU fimbrial promoters.

Expression of upstream predicted promoter region was assessed by β-galactosidase assay. Six growth conditions mimicking infection were evaluated. M63 broth (white bars) and agar (light grey bars) were tested as minimal media. LB broth (dark grey bars) and agar (black bars) were presented as rich media. Media inducing SPI-1 (wide striped bars) or -2 (slim striped bars) T3SS were used. Results are the mean of Miller units ± SEM of duplicate assays of biological triplicates.

2.2.4.2 Production and visualization of CU fimbriae in a S. Typhi afimbrial mutant

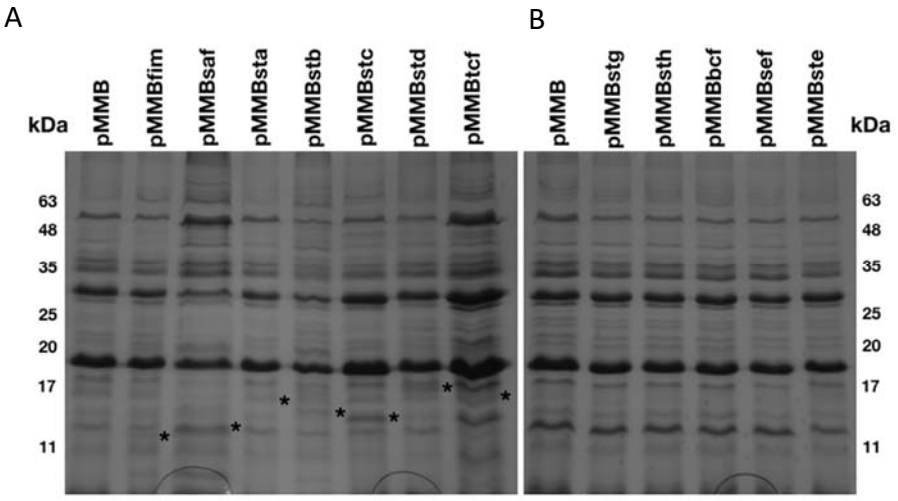

Figure 7. – Fimbrial major subunits in extracellular structures extract.

Bacteria were harvested from a LB agar plate supplemented with 50 μM IPTG and treated to extract the extracellular structures. (A) The control (pMMB) and 7 fimbrial gene clusters without pseudogene were migrated together on polyacrylamide gel and stained with Coomassie blue as well for (B) the control and the 5 fimbrial gene clusters without pseudogene. Asterisks are placed at the right of the proteinaceous bands considered as fimbrial subunits.

An afimbrial strain of *S.* Typhi was constructed in order to eliminate functional redundancy and assess the role of each individual fimbria. Markerless and non-polar deletions of each of the 14 fimbrial gene clusters of *S.* Typhi (CU, type IVb and curli fimbriae) were obtained by allelic exchange mutagenesis and result in an afimbrial strain harbouring a total deletion of 91.5 Kb from its genome. This strain had a similar growth curve compared to the wild-type strain (Annexes). Each complete fimbrial system was overexpressed by cloning on a IPTG-inducible vector and production of fimbrial proteins was induced in the afimbrial strain. The presence of the fimbrial subunits was verified on Coomassie blue stained gel (Figure 7). The production of the major subunits of Fim, Saf, Sta, Stb, Stc, Std and Tcf fimbrial proteins was confirmed by visualization of a band between 10 and 17 kDa that is consistent with predicted molecular mass of the mature secreted proteins. Mass spectrometry analyses identified StdA and StcA in the respective extracted bands. For Std, 53 specific peptides with 100% probability corresponding to StdA were identified, covering 167/193 amino acids, representing 87% of the predicted protein. For Stc, 43 specific peptides with 100% probability corresponding to StcA were identified, covering 131/176 amino acids, representing 74% of the predicted protein. When Fim fimbriae were induced, cells demonstrated a growth defect and lysis even at 10 µM IPTG (Annexes). No specific bands were visualized for any of the fimbrial gene clusters containing pseudogenes. Each fimbrial systems was induced for electronic microscopy analysis and the presence of Fim, Saf, Sta, Stb, Std and Tcf was confirmed as functionally assembled fimbriae were visualized on the bacterial surface (Figure 8). Fim fimbriae are straight and short. They cover most of the bacterial cell surface. Saf fimbriae are thin and aggregated together. Sta and Stb fimbriae are straight and long, but seem shorter than flagella. Std are short and thin fimbriae. Tcf fimbriae are cable-like fimbriae and intertwine together.

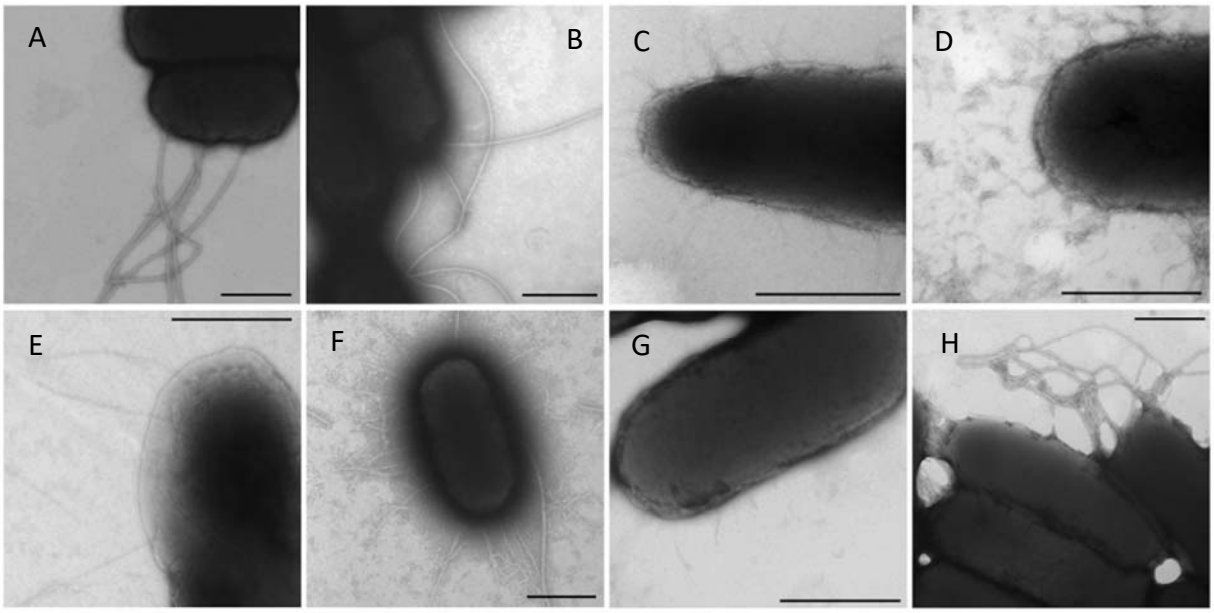

Figure 8. – Visible fimbriae in transmission electron microscopy.

Bacteria were harvested from a 24 hours-induction in LB broth supplemented with 1mM IPTG or on LB agar supplemented with 50µM IPTG and then fixated with 2% glutaraldehyde for 30 minutes. The formvar-carbon grids were stained with 1% phosphotungtic acid. Native operon was cloned under lactose-inducible promoter on pMMB207c vector and transformed into afimbrial ISP1820. (A-B) The controls (pMMB) present only flagella. (A) Afimbrial with pMMB207c. (B) WT strain with pMMB207c. (C-H) Fimbriae of different gene clusters are represented: (C) Fim fimbriae, (D) Saf fimbriae, (E) Sta fimbriae, (F) Stb fimbriae,(G) Std fimbriae and (H) Tcf fimbriae. Black bars = 500 nm.

2.2.4.3 Adhesion and invasion of epithelial cells

The role of fimbriae in adhesion to or invasion of intestinal epithelial INT-407 cells was evaluated. First, the effect of the deletion of a single fimbrial system was tested (Figure 9A). Deletion of *fim* and *sef* provoked a decrease in adherence. However, every fimbrial deletion caused a decrease in invasion to epithelial cells, only *Δbcf* and *Δste* mutants were not significant when compared to the wild-type. The addition of a single fimbrial system (under its native promoter) in the afimbrial strain (Figure 9B) was then tested. The addition of Fim, Saf, Sef, Stb, Stc or Ste caused a significant decrease in adherence and addition of any fimbrial system provoked a decrease in invasion of epithelial cells. Overall, fimbriae seem to have a generalized deleterious effect on invasion of epithelial cells, but variable effects on adherence.

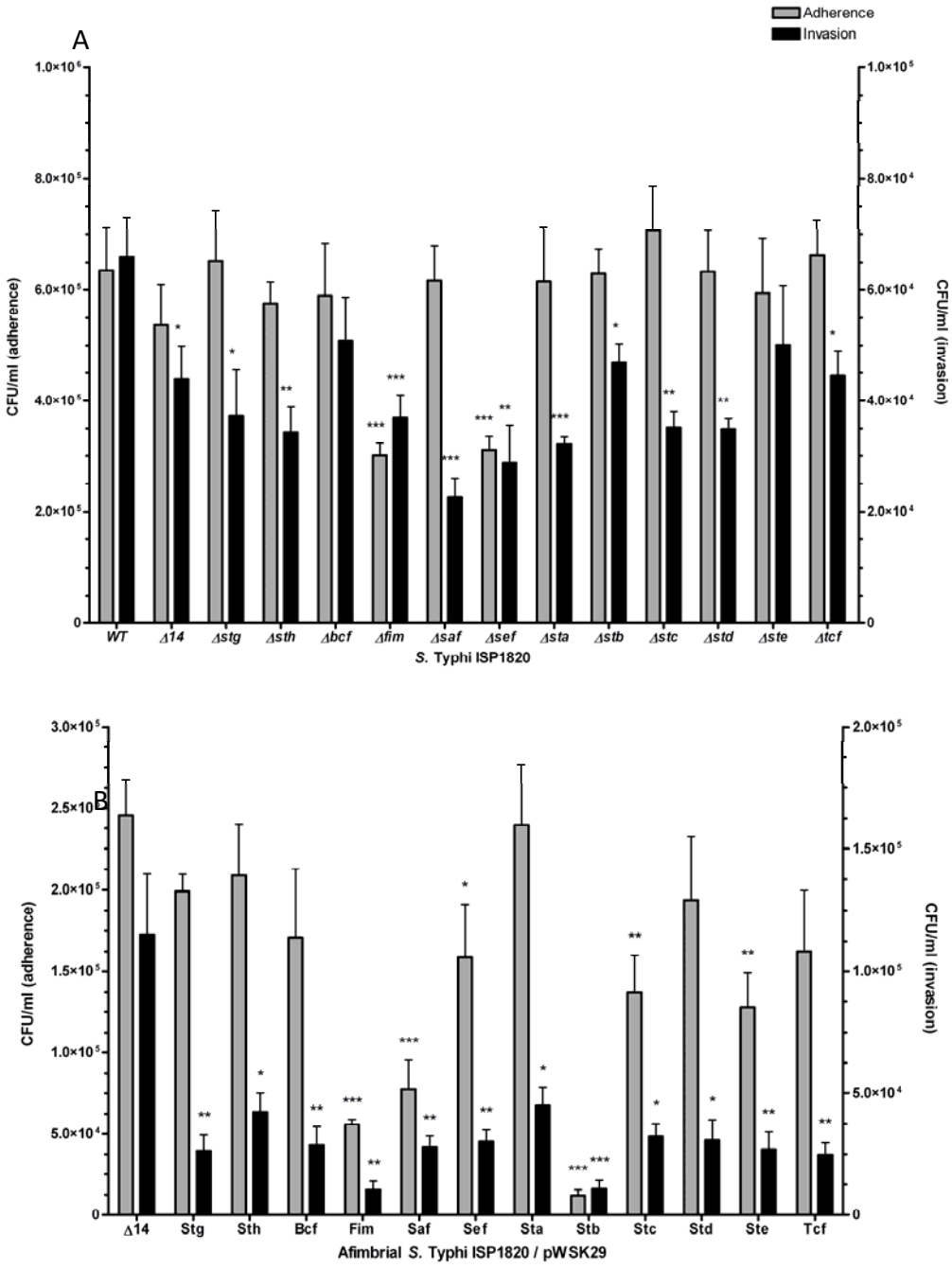

Figure 9. – Fimbrial interactions with INT-407 intestinal epithelial cells.

Adherence (gray bars) was determined after 90 minutes of infection. Gentamycin was added to medium for another 90 minutes (total of 180 minutes) to assess the invasion level (black bars). (A) Mutant strains with deletion of each fimbrial gene cluster were constructed and used for this assay. (B) Complete fimbrial gene cluster (including native promoter) was cloned on low-copy pWSK29 vector and transformed into afimbrial ISP1820. The results are presented as the mean ± SEM of the replicas.

2.2.4.4 Uptake and survival within macrophages

Interaction of *S.* Typhi with THP-1 macrophages was assessed for phagocytosis (t=0) and for survival after 24 hours (Figure 10A). Deletion of *stg* and *stb* significantly increased phagocytosis levels to 160 and 155% respectively while deletion of *bcf*, *saf* and *tcf* decreased it to 88, 65 and 87% respectively of the wild-type strain. Deletion of *bcf* increased survival to 126% of the wild-type strain, whereas deletion of *stc* and *std* resulted in decreased survival to 68 and 48% respectively compared to the wild-type strain. Regarding the addition of individual fimbria to the afimbrial strain, Fim and Stb present the most relevant phenotypes (Figure 10B): Fim fimbriae increased phagocytosis (259%) and survival (600%), whereas Stb decreased phagocytosis (10%) and survival (2%).

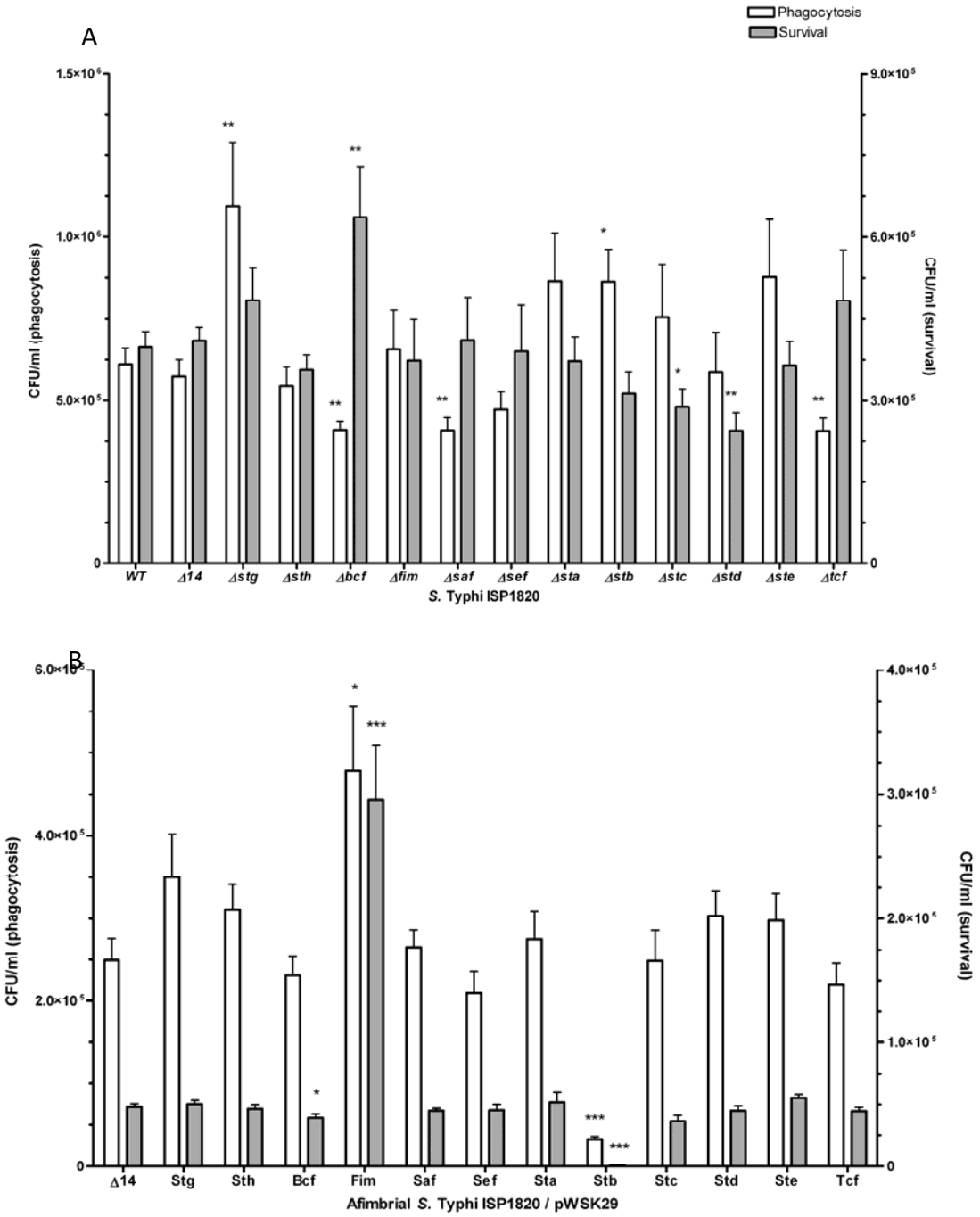

Figure 10. – Fimbrial interactions with THP-1 macrophages.

Phagocytosis (white bars) was measured after 20 minutes of infection. Gentamycin was added to medium overnight to assess the survival (grey bars). (A) Mutant strains with deletion of each fimbrial gene cluster were used for this assay. (B) Native promoter and operon was cloned on low-copy pWSK29 vector and transformed into afimbrial ISP1820. The results are presented as the mean ± SEM of the replicas.

2.2.4.5 Fimbriae and motility

The role of fimbriae on motility was tested on LB 0.3% agar plates. Either the deletion of one fimbrial system or the addition of individual fimbrial systems to the afimbrial strain did not show any significant phenotype compared to the wild-type control, as the same level of swimming motility was observed (Annexes). However, when each fimbrial system was overexpressed, the Fim fimbriae (pMMBfim) drastically decreased the swimming to 52.3 $\pm$ 3,2% of the control. By contrast, none of the other fimbrial systems showed a significant difference when compared to the control strain (Annexes).

2.2.4.6 Role of fimbriae on biofilm production

The role of fimbriae for biofilm formation was tested by crystal violet coloration assays. First, the consequence of the deletion of individual fimbrial systems was evaluated (Figure 11A). Deletion of *stg*, *bcf*, *saf* or *stc* decreased biofilm production to between 70 and 85% of the wild-type production. Introduction of each the individual fimbrial systems to the afimbrial strain was also evaluated (Figure 11B). Addition of Stg, Sth, Bcf and Ste reduced biofilm production to 72 to 88% of the control and addition of Stb increased biofilm production to 128%. We then tested the effect of fimbrial overexpression by induction of the fimbrial cluster (Figure 11C). Fim, Stc, Std and Tcf increased biofilm production to levels between 140 and 180% of the control strain, whereas Stg had decreased biofilm production to 68% (See also Figure 28).

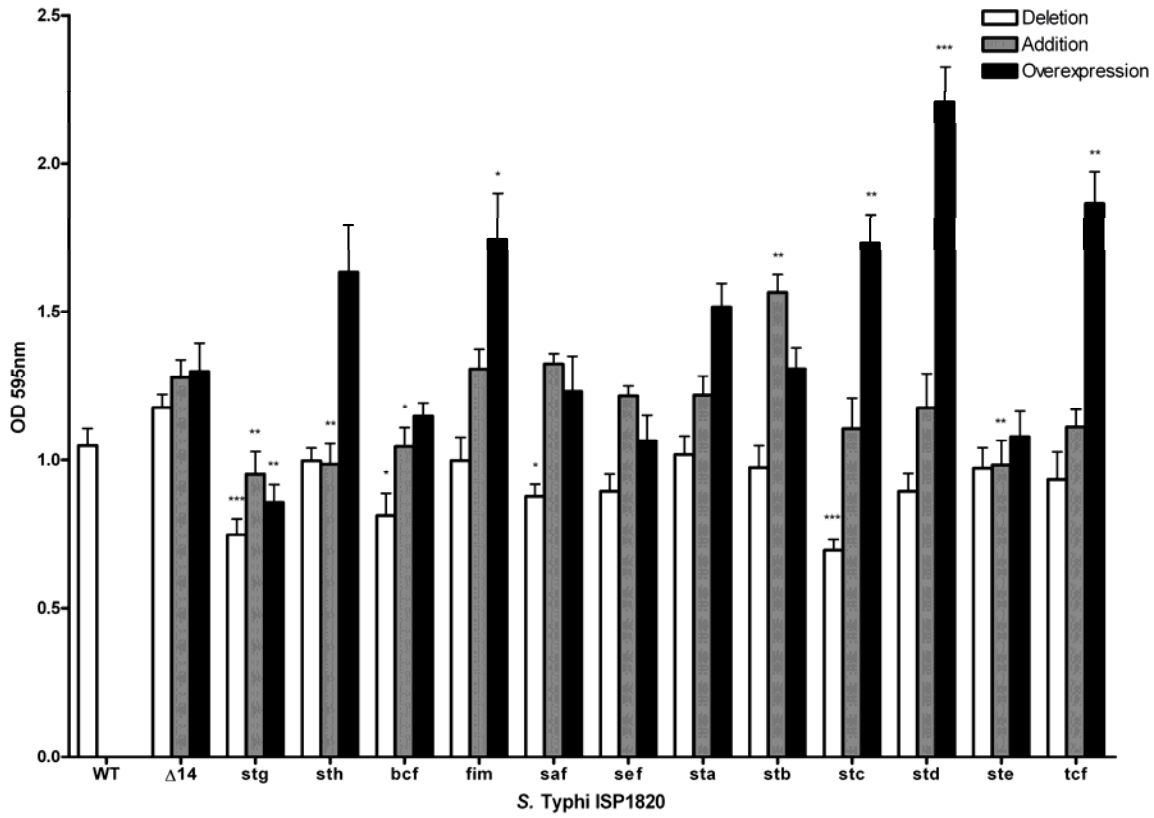

Figure 11. – Impact of fimbriae on biofilm formation.

Biofilm formation was performed with cholesterol-coated plate and bacteria were incubated statically for 72 hours in a bile-supplemented medium. Results are presented as the mean ± SEM of replicas. (White bars) Mutant strains with deletion of each fimbrial gene cluster were used for this assay. (Gray bars) Native promoter and operon was cloned on low-copy pWSK29 vector and transformed into afimbrial ISP1820. (Black bars) Native operon was cloned under lactose-inducible promoter on pMMB207c vector and transformed into afimbrial ISP1820.

2.2.5 Discussion

S. Typhi possesses 12 different chaperone/usher fimbriae that belong to 5 fimbrial clades (γ_1, γ_3, γ_4, π and α) based on usher homologies (Nuccio and Bäumler, 2007). These fimbriae were identified by whole-genome sequencing and most are considered to be putative, as it is not known under what conditions they may be expressed or they have not been characterized or visualized yet (Baker and Dougan, 2007). Here, we investigated the expression and characterization of all 12 of the *S.* Typhi CU fimbriae. We evaluated the expression of fimbrial promoters, production of fimbrial proteins and functional production of fimbriae. We also characterized the effects of fimbrial deletions or phenotypes due to introduction of specific fimbrial gene clusters to an afimbrial strain on interaction with host epithelial cells and macrophages, bacterial motility, and biofilm formation.

Fimbriae are poorly expressed when grown under laboratory conditions. A study from Kröger et al. (2013) compared 22 infection-mimicking conditions by an RNA-seq-based analysis of *S.* Typhimurium SL1344 and revealed that fimbrial genes, including the 7 CU fimbrial operons in common with *S.* Typhi, were not or are very poorly expressed. To determine the expression of *S.* Typhi CU fimbrial promoters, the use of a multi-copy reporter gene fusion (pRS415) with each fimbrial promoter was required to obtain a sufficient β-galactosidase activity, as a chromosomal *lacZ* fusion did not provide a sufficient level of expression for quantification of Miller units (data not shown). Six growth conditions were tested: rich (LB), minimal (M63), both in liquid (broth) and solid (agar), and SPI-1 and -2 T3SSs induction conditions (Figure 6). As the niche of *S.* Typhi is restricted to the human body, only 37°C was tested. Interestingly, one specific fimbrial promoter from each of the different CU clades was dominant (*sth* for γ_1, *saf* for γ_3, *stc* for γ_4 and *std* for π), suggesting a role for each fimbrial clade in *S.* Typhi. Also, the majority of the fimbrial systems had a unique expression pattern (except for stg and ste (Tableau 4). The highest expression for each fimbrial promoter was obtained during growth in minimal medium. Low nutrient conditions may be encountered by *S.* Typhi during colonization of liver and spleen, in the blood, or in the gall bladder, corresponding to different sites where fimbriae may be needed (Gonzalez-Escobedo et

al., 2011; Keestra-Gounder et al., 2015). SPI-1 T3SS-inducing condition was usually the condition with the lowest expression, except for *stc* and *std* which demonstrated their lowest on M63 agar. In *S.* Typhimurium, the balance between adherence (fimbriae), invasion (SPI-1 T3SS) and motility (flagella) is usually finely regulated. The induction of one of these elements can therefore result in a decrease in expression of the other factors (Clegg and Hughes, 2002; Saini et al., 2010; Baxter and Jones, 2015). Otherwise, other SPI-1 inducing conditions might be more optimal for the expression of the studied fimbriae and might reflect more accurately interaction with epithelial cells.

As fimbriae are poorly expressed in laboratory conditions, the functional assembly of putative fimbrial operons of *S.* Typhi was induced and detection of the major subunit proteins were visualized for Fim, Saf, Sta, Stb, Stc, Std, and Tcf systems. No specific bands were observed for putative fimbrial gene clusters that contain pseudogenes. This could be explained by lack of functional assembly for these fimbriae. Fim, Saf, Sta, Stb, Std, and Tcf fimbriae were also visualized by TEM and demonstrated differences in morphology and distribution on the surface of cells which may imply difference in pathogenesis functions (Figure 8). Fimbrial gene clusters with pseudogenes (*stg*, *sth*, *bcf*, *sef,* and *ste*) and Stc fimbriae were not observed by TEM. This can be due to their detachment during the grid preparation or to the absence of functional assembly. Most of the pseudogenes are located in usher or subunits genes that may prevent the formation of the fimbriae by avoiding the transport of the subunits through the outer-membrane or the fimbriae formation. However, fimbrial operons with pseudogenes may be functional by complementing the non-functional usher by another fimbrial usher or by using a suppressor tRNAs to bypass stop codon to form a functional usher or subunit (Berrocal et al., 2015). The presence of pseudogenes may still impact on pathogenesis as some of the fimbrial proteins may be exported in the environment or fixed to the surface in absence of full-length fimbriae, acting similarly to afimbrial adhesin.

The potential roles of the different CU fimbriae were investigated by testing effects of the individual deletion of these systems from the wild-type strain or by introduction of each system to an afimbrial *S.* Typhi strain on bacterial interactions with host cells (INT-407 and THP-1) (Figures 9–10, Tableau 4), on motility, and on biofilm formation (Figure 11). Interestingly, each fimbria seems to have a role in invasion as deletion of one of them or individual addition of fimbriae in afimbrial strain decrease invasion to epithelial cells compared to their control. This implies that fimbriae may affect invasion by regulation of the SPI-1 T3SS or that they are critical for the initial contact with host cells to allow a stable interaction between the T3SS and the host. A combination of multiple fimbriae may be important for the optimal contact between the bacteria and the epithelial cells. Another hypothesis is that the SPI-1 T3SS or other adhesins at the surface of the bacteria are affected by the deletion of fimbriae and may confer lower invasion of epithelial cells. The functionality of the T3SS may be verified by a secretion assay in further studies and the presence of different adhesins could be determined by Western Blot. There was no difference in motility, except for induction of Fim, whereas most of fimbriae had variable effects (positive or negative) on biofilm formation, except for Sef and Sta. Specific results for each fimbrial system are discussed in the next sections.

Tableau 4. – Summary of fimbrial expression and pathogenesis phenotypes.

Fimbriae	Promoter expression						INT-407 infection				THP-1 infection				Motility	Biofilm formation		
							Adherence		Invasion		Uptake		Survival					
	M63 broth	M63 agar	LB broth	LB agar	SPI-1	SPI-2	Deletion	Addition	Deletion	Addition	Deletion	Addition	Deletion	Addition	Overexpression	Deletion	Addition	Overexpression
Stg	**1**	**5**	**2**	**3**	**6**	**4**	0	0	-	-	+	0	0	0	0	-	-	-
Sth	**1**	**4**	**3**	**2**	**6**	**5**	0	0	-	-	0	0	0	0	0	0	-	0
Bcf	**1**	**3**	**4**	**2**	**6**	**5**	0	0	0	-	-	0	+	-	0	-	-	0
Fim	**1**	**3**	**2**	**4**	**6**	**5**	-	-	-	-	0	+	0	+	-	0	0	+
Saf	**1**	**5**	**2**	**4**	**6**	**3**	0	-	-	-	-	0	0	0	0	-	0	0
Sef	**1**	**4**	**3**	**5**	**6**	**2**	-	-	-	-	0	0	0	0	0	0	0	0
Sta	**1**	**5**	**2**	**4**	**6**	**3**	0	0	-	-	0	0	0	0	0	0	0	0
Stb	**1**	**3**	**2**	**5**	**6**	**4**	0	-	-	-	+	-	0	-	0	0	+	0
Stc	**1**	**6**	**2**	**5**	**4**	**3**	0	-	-	-	0	0	-	0	0	-	0	+
Std	**1**	**6**	**2**	**3**	**4**	**5**	0	0	-	-	0	0	-	0	0	0	0	+
Ste	**1**	**5**	**2**	**3**	**6**	**4**	0	-	0	-	0	0	0	0	0	0	-	0
Tcf	**1**	**5**	**3**	**4**	**6**	**2**	0	0	-	-	-	0	0	0	0	0	0	+

SPI-1 = SPI-1 T3SS induction; **SPI-2** = SPI-2 T3SS induction; **Deletion** = mutant strains;

Addition = afimbrial strain with pWSK29 vector; **Overexpression** = afimbrial strain with pMMB207c vector;

1 to **6** = descending order of expression; **0** = no effect; **-** = deleterious effect; **+** = increasing effect

The highest expressed promoter was *saf*. Saf fimbriae were also visualized on TEM. This fimbria is conserved among *S. enterica* subspecies *enterica* (ssp. I). No noticeable phenotype during cell infection, motility or biofilm formation has been previously reported (Folkesson et al., 1999; Humphries et al., 2003). However, *S.* Typhi Saf fimbriae were strongly expressed inside human macrophages (Faucher et al., 2006). The main effect observed for the *saf* deletion mutant of ISP1820 was decreased phagocytosis, invasion and biofilm formation (Tableau 4). Also, the addition of the *saf* gene cluster to the afimbrial strain decreased adherence and invasion to epithelial cells. In summary, Saf fimbriae are the most expressed fimbriae in *S.* Typhi and demonstrated few differences in the aspects of pathogenesis.

Std and Stc are highly expressed and the only systems with an expression level in SPI-1 T3SS inducing conditions that was not their lowest (Tableau 4). They also share similarities in infection of host cells, with a decrease of survival in macrophages, and in biofilm formation when overexpressed. Std and Stc fimbriae are widely distributed in *Salmonella enterica* ssp. I, forming the core fimbriae with Csg (curli fimbriae), Fim, Sth, Bcf and Stb (Dufresne et al., 2017). The role of these two fimbriae for invasion and survival could then be generalized to other serovars of *S. enterica* ssp. I. Interestingly, *S.* Typhimurium both Std and Stc fimbriae were recognized to be involved in intestinal persistence in mice (Weening et al., 2005). The role of these two fimbriae for invasion and survival could be generalized to other serovars of *S. enterica* ssp. I and their affinity could be for intestinal epithelial cells.

sth demonstrated a high level of expression similar to levels of *stc* expression. Sth is highly expressed when grown on LB agar, similarly to *bcf*, which could suggest a role for these fimbriae on solid surfaces (Tableau 4). However, Sth did not contribute significantly to adhesion to or interaction with human cells, whereas the deletion of *bcf* caused a decrease in phagocytosis, but an increase in survival in macrophages. Addition of *sth* or *bcf* gene clusters reduced biofilm formation, suggesting that their role is not in interbacterial adhesion but may be important in adherence to other host cells or in environmental conditions. These two fimbriae are present in

most serovars of *S. enterica* ssp. I, and also belong to the fimbrial core. However, as *S.* Typhi is a pathogen with a restricted niche, the presence of pseudogenes in those two gene clusters may partially explain why the bacterium is human-specific. Also, these two fimbriae were not visualized in TEM. However, the truncated ushers may allow the export of fimbrial proteins in extracellular environment, which may explain the effects on pathogenesis presented by Sth and Bcf fimbriae.

stg and *ste* promoters exhibited the same pattern of expression but not at same level, *stg* expression was moderate whereas *ste* expression was lower (Tableau 4). Deletion of *stg* from *S.* Typhi ISP1820 increased phagocytosis in macrophages. This is consistent with Forest et al. (2007) as they used *S.* Typhi ISP1820 and THP-1 macrophages as well. Berrocal et al. (2015), however obtained results that loss of *stg* decreased phagocytosis, but they used *S.* Typhi STH2370, a Chilean isolate, and a different macrophage cell line. Stg was also found to have deleterious effects on biofilm formation, regardless of its deletion, addition or overexpression. The deleterious effect of the deletion of *stg* suggests that this fimbria is involved in biofilm formation. However, the addition or the overexpression of Stg in the afimbrial strain suggests that other fimbriae must modulate action of Stg and are required for establishing a mature biofilm. Addition of the Ste fimbriae also reduced biofilm formation. These two fimbriae have pseudogenes in their operon and are not present in *S.* Typhimurium. Despite the presence of pseudogenes and the absence of fimbrial assembly suggesting these gene clusters may not be functional, these fimbrial systems contribute during bacterial interactions with host cells and biofilm formation.

Fim and Stb fimbriae had poor expression in any growth conditions tested. However, they showed major phenotypes in most of the assays. Deletion of *fim* decreased adherence and invasion to epithelial cells, while addition of Fim to the afimbrial strain also decreased adherence and invasion to INT-407 cells. When added to the afimbrial strain, Fim increased phagocytosis and survival in macrophages. Different studies involved FimH, the adhesin of Fim fimbriae, as an important marker of host specificity. For S. Typhi, the presence of a valine in position 223 of FimH

seems to be determinant for the binding of human cell lines (Kisiela et al., 2012; Yue et al., 2015). The defect in swimming may be due by the decrease in growth rate or by overexpression of the Fim fimbriae. Fim fimbriae may be a major player of S. Typhi pathogenesis and may regulate other fimbriae and virulence factors like flagella, LPS or T3SSs. Stb may be involved in long-term persistence by biofilm formation and may regulate negatively early stages of infection like host cell interactions. The balance between motility, invasion and persistence is critical for a successful infection, and Stb seems to inhibit invasion in favor of persistence. The inhibition of interaction with macrophages by Stb fimbriae could be physical: Stb fimbriae are long and cover the majority of the surface of the bacteria, which may interfere with recognition molecules for receptors on macrophages, and influence the interaction with host cells. Stb and Fim may have opposing roles on macrophages/bacteria interaction and, in a wild-type strain, may counteract the action of each other at different steps to lead to a successful infection.

Expression of the tcf promoter was low in every condition tested. However, Tcf fimbriae were previously visualized and are similar to cable (Cbl) pili of *Burkholderia cepacia*, which are in the same CU clade (α) and share more genetic homologies than other members of the α clade (Leclerc et al., 2016). Deletion of *tcf* decreased phagocytosis by macrophages, but does not affect survival. Overexpression of Tcf fimbriae increased biofilm formation. Tcf may play a role in late stages of infection, from the interaction with macrophages to persistence in gall bladder.

Deletion of the *sef* gene cluster decreases adherence and invasion of epithelial cells. However, its presence in an afimbrial strain did not allow recovery of the control levels. Despite the presence of a pseudogene in its operon, *sef* influenced epithelial cell interactions.

Overall, CU fimbriae act as redundant systems on different aspects of *S.* Typhi pathogenesis, such as bacteria/host cell interactions and biofilm formation. In order to reflect more accurately the *S.* Typhi pathogenesis, fimbriae should be also studied in interactions with other host cells targets such as splenocytes, hepatocytes, and cells from the gall bladder. Some fimbriae are similar in their functions and usually have reverse level of expression in a matter of balanced expression

(Figure 12). The similarity between certain fimbriae could be a form of functional redundancy. Fimbrial production is energetically demanding for the bacteria and the balance of expression between few fimbriae of similar function and presence of pseudogenes could be a way to prevent unnecessary redundancy and preserve *S.* Typhi energy. A tightly regulated equilibrium between stages of pathogenicity of *S.* Typhi is important for a successful infection of the host. The presence of fimbriae at the surface of the bacteria is confirmed for Fim, Saf, Sta, Stb, Std, and Tcf. Each of these fimbriae affect invasion of epithelial cells and phagocytosis by macrophages, although Std only affected survival in macrophages. Fimbriae such as Stc and gene clusters containing pseudogenes (*bcf*, *stg*, *ste,* and *sef*) also contributed to different aspects of infection based on our investigation. Each of these systems had an impact on interactions with epithelial cells, although *sef* and *ste* did not alter macrophage interactions. Further, they all had an effect on biofilm formation, except for *sef* and *sta*. Sta and Sth do not present particular function in the case of *S.* Typhi. Sta could be involved in a process of human-specificity, as it is only present in host-restricted strains. For Sth, it may lose its function in *S.* Typhi as it could have an importance in adhesion to abiotic surfaces in other serovars. Fimbriae are critical components of the equilibrium between stages of pathogenesis and further research is needed to more fully understand their complex role for *S.* Typhi and the pathogenesis of typhoid fever.

2.2.6 Authors contributions

KD and FD designed the research; KD and JS proceed to the experiments; KD and FD analyzed the data; KD drafted the manuscript; KD, FD and JS revised the manuscript.

2.2.7 Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.2.8 Acknowledgments

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Tableau 5. – Bacterial strains and plasmids used for this study

Strain or plasmid	Name	Characteristic	Source or reference
		***S.* Typhi**	
DEF1045	WT	ISP1820 wild-type	R. Curtiss III, U. Florida
DEF004	*Δstg*	WT Δ*stgABCD*	(Forest *et al.*, 2007)
DEF621	*Δsth*	ISP1820 Δ*sthABCDE*	This study
DEF563	*Δbcf*	ISP1820 Δ*bcfABCDEFG*	This study
DEF154	*Δfim*	ISP1820 Δ*fimAICDHFZYXW*	This study
DEF084	*Δsaf*	ISP1820 Δ*safAEBCD*	This study
DEF605	*Δsef*	ISP1820 Δ*sefABCD*	This study
DEF044	*Δsta*	ISP1820 Δ*staABCDEFG::kan*	This study
DEF564	*Δstb*	ISP1820 Δstb*ABCDE*	(Sabbagh *et al*., 2012)
DEF602	*Δstc*	ISP1820 Δ*stcABCD*	(Sabbagh *et al*., 2012)
DEF600	*Δstd*	ISP1820 Δ*stdABC*	This study
DEF603	*Δste*	ISP1820 Δ*steABCDEF*	This study
DEF441	*Δtcf*	ISP1820 Δ*tcfABCD*	(Leclerc *et al.*, 2016)
DEF664	Afimbrial ISP1820	ISP1820 Δ*bcfABCDEFG* Δ*csgCABDEFG* Δ*fimAICDHFZYXW* Δ*pilLMNOPQRSTUVK* Δ*safAEBCD* Δ*sefABCD* Δ*staGFEDCBA::kan* Δ*stbEDCBA* Δ*stcDCBA* Δ*stdCBA* Δ*steABCDEF* Δ*stgABCD* Δ*sthEDCBA* Δ*tcfABCD*	This study

DEF1172	Afimbrial ISP1820 Kan^S	ISP1820 Δ*bcfABCDEFG* Δ*csgCABDEFG* Δ*fimAICDHFZYXW* Δ*pilLMNOPQRSTUVK* Δ*safAEBCD* Δ*sefABCD* Δ*staABCDEFG* Δ*stbABCDE* Δ*stcABCD* Δ*stdABC* Δ*steABCDEF* Δ*stgABCD* Δ*sthABCDE* Δ*tcfABCD*	This study
DEF1153	pRS	WT (pRS415)	This study
DEF1125	pRSstg	WT (pSIF469)	This study
DEF1124	pRSsth	WT (pSIF470)	This study
DEF1130	pRSbcf	WT (pSIF471)	This study
DEF1095	pRSfim	WT (pSIF474)	This study
DEF1090	pRSsaf	WT (pSIF472)	This study
DEF1128	pRSsef	WT (pSIF473)	This study
DEF1082	pRSsta	WT (pSIF464)	This study
DEF1127	pRSstb	WT (pSIF465)	This study
DEF1126	pRSstc	WT (pSIF466)	This study
DEF992	pRSstd	WT (pSIF467)	This study
DEF1131	pRSste	WT (pSIF468)	This study
DEF1132	pRStcf	WT (pSIF219)	This study
DEF1019	pMMB	Afimbrial ISP1820 (pMMB207c)	This study
DEF1106	pMMBstg	Afimbrial ISP1820 (pSIF397)	This study
DEF1133	pMMBsth	Afimbrial ISP1820 (pSIF416)	This study
DEF1113	pMMBbcf	Afimbrial ISP1820 (pSIF395)	This study
DEF1151	pMMBfim	Afimbrial ISP1820 Kan^S (pSIF429)	This study
DEF1114	pMMBsaf	Afimbrial ISP1820 (pSIF412)	This study

DEF1155	pMMBsef	Afimbrial ISP1820 (pSIF432)	This study
DEF1111	pMMBsta	Afimbrial ISP1820 Kan^S (pSIF413)	This study
DEF1107	pMMBstb	Afimbrial ISP1820 (pSIF414)	This study
DEF1112	pMMBstc	Afimbrial ISP1820 (pSIF424)	This study
DEF1207	pMMBstd	Afimbrial ISP1820 Kan^S (pSIF427)	This study
DEF1108	pMMBste	Afimbrial ISP1820 (pSIF396)	This study
DEF1020	pMMBtcf	Afimbrial ISP1820 (pSIF420)	This study
DEF1192	pWSK	Afimbrial ISP1820 Kan^S (pWSK29)	This study
DEF1185	pWSKstg	Afimbrial ISP1820 Kan^S (pSIF026)	This study
DEF1223	pWSKsth	Afimbrial ISP1820 Kan^S (pSIF450)	This study
DEF1228	pWSKbcf	Afimbrial ISP1820 Kan^S (pSIF458)	This study
DEF1224	pWSKfim	Afimbrial ISP1820 Kan^S (pSIF451)	This study
DEF1191	pWSKsaf	Afimbrial ISP1820 Kan^S (pSIF036)	This study
DEF1190	pWSKsef	Afimbrial ISP1820 Kan^S (pSIF444)	This study
DEF1184	pWSKsta	Afimbrial ISP1820 Kan^S (pSIF046)	This study
DEF1187	pWSKstb	Afimbrial ISP1820 Kan^S (pSIF232)	This study
DEF1221	pWSKstc	Afimbrial ISP1820 Kan^S (pSIF445)	This study
DEF1227	pWSKstd	Afimbrial ISP1820 Kan^S (pSIF457)	This study
DEF1222	pWSKste	Afimbrial ISP1820 Kan^S (pSIF446)	This study
DEF1188	pWSKtcf	Afimbrial ISP1820 Kan^S (pSIF119)	This study
DEF1235	WT/pWSK	WT (pWSK29)	This study
DEF1236	WT/pWSKfim	WT (pSIF451)	This study

DEF1232	Δfim/pWSKfim	*Δfim* (pSIF451)	This study
		***E.* coli**	
DEF1162	MGN-617	SM10 λpir asd thi thr leu tonA lacY supE recA RP4 2-Tc : :Mu[λpir] *asdA4*	(Kaniga *et al*., 1998)
		Plasmids	
pMEG-375		*sacRB mobRP4 oriR6K*, Cm^r Ap^r	R. Curtiss III, U. Florida
pSIF004		pMEG-375 with flanking region of *stg* operon used for *stg* operon deletion	(Forest *et al.*, 2007)
pSIF210		pMEG-375 with flanking region of *sth* operon used for *sth* operon deletion	This study
pSIF175		pMEG-375 with flanking region of *bcf* operon used for *bcf* operon deletion	(Sabbagh et *al.*, 2012)
pSIF064		pMEG-375 with flanking region of *fim* operon used for *fim* operon deletion	This study
pSIF034		pMEG-375 with flanking region of *saf* operon used for *saf* operon deletion	This study
pSIF206		pMEG-375 with flanking region of *sef* operon used for *sef* operon deletion	This study
pSIF046		pMEG-375 with flanking region of *sta* operon used for *sta* operon deletion	This study
pSIF176		pMEG-375 with flanking region of *stb* operon used for *stb* operon deletion	(Sabbagh et *al.*, 2012)
pSIF198		pMEG-375 with flanking region of *stc* operon used for *stc* operon deletion	(Sabbagh et *al.*, 2012)

pSIF202	pMEG-375 with flanking region of *std* operon used for *std* operon deletion	This study
pSIF203	pMEG-375 with flanking region of *ste* operon used for *ste* operon deletion	This study
pSIF098	pMEG-375 with flanking region of *tcf* operon used for *tcf* operon deletion	(Leclerc *et al*., 2016)
pRS415	Multicopy vector with a promotorless, *lacZ* reporter gene, Ap^r	(Simons *et al.*, 1987)
pSIF469	pRS415 carrying the promoter region of *stgA*	This study
pSIF470	pRS415 carrying the promoter region of *sthA*	This study
pSIF471	pRS415 carrying the promoter region of *bcfA*	This study
pSIF474	pRS415 carrying the promoter region of *fimA*	This study
pSIF472	pRS415 carrying the promoter region of *safA*	This study
pSIF473	pRS415 carrying the promoter region of *sefA*	This study
pSIF464	pRS415 carrying the promoter region of *staA*	This study
pSIF465	pRS415 carrying the promoter region of *stbA*	This study
pSIF466	pRS415 carrying the promoter region of *stcA*	This study
pSIF467	pRS415 carrying the promoter region of *stdA*	This study
pSIF468	pRS415 carrying the promoter region of *steA*	This study
pSIF219	pRS415 carrying the promoter region of *tcfA*	(Leclerc et al., 2016)
pMMB207c	Wide host range vector, IPTG-inducible	(Morales *et al.*, 1991)
pSIF397	pMMB207-*stgABCD*	This study
pSIF416	pMMB207-*sthABCDE*	This study

pSIF395	pMMB207-*bcfABCDEFG*	This study
pSIF429	pMMB207-*fimAICDHFZYXW*	This study
pSIF412	pMMB207-*safAEBCD*	This study
pSIF432	pMMB207-*sefABCD*	This study
pSIF413	pMMB207-*staABCDEFG*	This study
pSIF414	pMMB207-*stbABCDE*	This study
pSIF424	pMMB207-*stcABCD*	This study
pSIF427	pMMB207-*stdABCD*	This study
pSIF396	pMMB207-*steABCDEF*	This study
pSIF420	pMMB207-*tcfABCD*	(Leclerc et al., 2016)
pWSK29	Low copy number cloning vector, Ap^r	(Wang *et al.*, 1991)
pSIF026	pWSK29 carrying a 5.4 kb fragment of *stgABCD*	(Forest *et al.*, 2007)
pSIF450	pWSK29 carrying a 6 kb fragment of *sthABCDE*	This study
pSIF458	pWSK29 carrying a 7.4 kb fragment of *bcfABCDEFG*	This study
pSIF451	pWSK29 carrying a 9.7 kb fragment of *fimAICDHFZYXW*	This study
pSIF036	pWSK29 carrying a 4.6 kb fragment of *safAEBCD*	This study
pSIF444	pWSK29 carrying a 5 kb fragment of *sefABCD*	This study
pSIF046	pWSK29 carrying a 8 kb fragment of *staABCDEFG*	This study
pSIF232	pWSK29 carrying a 7.1 kb fragment of *stbABCDE*	This study
pSIF445	pWSK29 carrying a 5.2 kb fragment of *stcABCD*	This study
pSIF457	pWSK29 carrying a 5.8 kb fragment of *stdABCD*	This study
pSIF446	pWSK29 carrying a 6.7 kb fragment of *steABCDEF*	This study

pSIF119	pWSK29 carrying a 5.8 kb fragment of *tcfABCD*	(Leclerc et al., 2016)

Tableau 6. – Primers used in this study

Primers	**Sequence (5'-3')***
STGA-F	CGGGATCCGAGATGAGAATAACGGAATA
STGA-R	AACTGCAGCCAGCAAATGCCGTTTTGTT
STGD-F	*AA*CTGCAGGCCGCAGAGCTGTGAAATTG
STGD-R	GCTCTAGACATTGATATGACTTATTTTG
SthA F1	CGGGATCCCGTAGAATAGCCGCCTGCTT
SthA R2	*CCGTCAGTTT*TGTGGATGCTACGGCAGTTA
SthE F3	*GCATCCACA*AACTGACGGCATCACATTTTC
SthE R4	GCTCTAGAGCCATCTGGACTGGTATTCG
Bcf F1	CGGGATCCACTCACGACGTTGAGTAGCT
Bcf R2	*ACGTCATTC*TGACGGTTGTAGTATCCGCT
Bcf F3	*CAACCGTCA*GAATGACGTGGGAACCTTAG
Bcf R4	AAGGAAAAAAGCGGCCGCTGCTACGCGGTTCAGTCATA
FimAF	CGGGATCCGATATCGAAACCGGGTGTGT
FimAR-over	*ACCTACAGG*GCATATTGGTGCCTTC
FimWR-over	*ATACGCTGC*CCTGTAGGTATCGTTACT
Fim WF	GCTCTAGACTCAGCACGCATAAAGTG
SafA-F	CGGGATCCGACTGGCATTCTATTCCACCCTG
SafA-R	*GTGGACCTG*CAGTTATCATGCTCAACACGCTTG

SafD-F	*TGATAACTG*CACCTGGACGCTGGATTTTAAG
SafD-R	GCTCTAGACCCGCTCACAAAAGAGTGAATC
SefA F1	CGGGATCCGCATCCGCACAGATAAATTG
SefA R2	*GTCTTCTCC*CTGCCTGAACCTCTGCTTTG
SefD F3	*TTCAGGCAG*GGAGAAGACTGGCAACCAGA
SefD R4	GCTCTAGAATGCCGTAGGAATGTCAAGC
StaAF	AACTGCAGGCGTGAAGAGGAAACTACTT
StaAR	GCTCTAGAAAATAATCATGGAACCAGTC
StaGF	AACTGCAGTTATCCAAAGCAGATTATGG
StaGR	CGGGATCCGCGTTAGCCTTTTCTGGGCA
Stb F1	CGGGATCCTGCTGAATTCTGGCCTGTCT
Stb R2	*GTTATTGCC*CGCCGAAAACAGCACTTGAT
Stb F3	*TTTTCGGCG*GGCAATAACACGACGGGTTT
Stb R4	AAGGAAAAAAGCGGCCGCCAGGAGGGTATAGCTCACAT
STY2378-81 F1 (*stc*)	GCTCTAGATGTTGACT GCCTTCACTA CC
STY2378-81 R2 (*stc*)	*GCTGAAATT*AAGCGACTGCGCTGATCTAT
STY2378-81 F3 (*stc*)	*CAGTCGCTT*AATTTCAGCGGTGTTCGTAC
STY2378-81 R4 (*stc*)	AAGGAAAAAAGCGGCCGCGCGATAACTTCCTGTCTATG
StdA F1	CGGGATCCCGATGGAAAGTTCAGGTGCT
StdA R2	*TTAAGGGCA*CCGCCATGGCAAGTATTATT
StdC F3	*CCATGGCGG*TGCCCTTAAAGGCTGTTCTG

StdC R4	GCTCTAGAATACCTGGCTCAACCGCATA
SteA F1	CGGGATCCCTATGCCGCATATCCCTTGA
SteA R2	*CTCTGCCAA*CCGGAGACAATTCCCATAAC
SteD F3	*TGTCTCCGG*TTGGCAGAGGGAAATACCAT
SteD R4	GCTCTAGACCAGAGCATCAATGCCTTT
TcfAF prom	GCTCTAGACATGATGATCAGTCTATTTGTGGC
TcfAR over	*TGTCAGGGT*AATTTCTGCCGCCATGGGATA
TcfDF over	*GCAGAAATT*ACCCTGACAACACAACCCTT
TcfDR	AAGGAAAAAAGCGGCCGCAGCAGAACCTCACGCATTGA
Stg_Prom_F_EcoRI	GCGAATTCCGGGAGATGAGAATAACGGA
Stg_Prom_R_BamHI	GTGGATCCAGTAGAAGACAGAACCAGAGCG
Sth_Prom_F_EcoRI	GCGAATTCAAATCCAGTCATCTACCGTACTTC
Sth_Prom_R_BamHI	GCGGATCCGTGGATGCTACGGCAGTTAACA
Bcf_Prom_F_Short_EcoRI	GTGGATTCAACTCACGACGTTGAGTAGCTG
Bcf_Prom_R_BamHI	GCGGATCCCAACATTCCGCCAAAGGCAA
Fim_Prom_F_Long_EcoRI	AGGAATTCCTTCAAGTCAAAGGGGATAACGCT
Saf_Prom_F_EcoRI	CAGAATTCTGTTATTACCAGCCAGGGAT
SafA R2	GTCCAGGTGCAGTTATCATGCTCAACACGCTTG
Sef_Prom_F_EcoRI	GCGAATTCCTTAGTCGCATGTCCACTCTTGCT
Sef_Prom_R_BamHI	ACGGATCCTGGGCACTGCCACATGCAATTA
Sta_Prom_F_EcoRI	GCGAATTCCAGCCGCTTAATGCAATTAAACA

Sta_Prom_R_BamHI	ATGGATCCTGCGGCAGCTAAAATCGCTT
Stb_Prom_F_Short_EcoRI	GCGAATTCCATCGGGAGGTTTAACTGATACGG
Stb_Prom_R_BamHI	GCGGATCCGCGAGCCTGTGATCATTGCTAAATA
Stc_Prom_F_EcoRI	GCGAATTCAATTCCGCAGGCCCATATCA
Stc_Prom_R_BamHI	GCGGATCCCCCAGCGCTCATAAATACAGCA
Std_Prom_F_EcoRI	GCGAATTCGTTCTTTGCTGTCGGCATTG
Std_Prom_R_BamHI	AAGGATCCACAGAAGCGCCATACATCATACCG
Ste_Prom_F_2_EcoRI	GCGAATTCACAGGTGTTGAATGCTACCTTTCCC
Ste_Prom_R_BamHI	GCGGATCCGCCGGAGACAATTCCCATAACTAAA
TcfAR prom	AATTTCTGCCGCCATGGGATA
Stg_Operon_F_SacI	GCGAGCTCAGGAAACAGACCATGAAACTGAATTTAATTGC
Sth_Operon_F_BamHI	GCGGATCCAGGAAACAGACCATGTTTAATAAGAAAATTATCA
Bcf_Operon_F_SacI	CGGAGCTCACCAAACAGACCATGAAAAAGCCTGTACTAGCA
Bcf_Operon_R_XbaI	GCTCTAGACGTTCGATAAGATCGGAAAG
Fim_Operon_F2_BamHI	GCGGATCCAGGAAACAGACCATGAAACATAAATTAATGACC
Fim_Operon_R3_XbaI	GCTCTAGATCAGGCACTCCTGAGTCAAT
Saf_Operon_F_EcoRI	GCGAATTCAGGAAACAGACCATGAAAAACATAAAAAAAT
Saf-RC	GCTCTAGATGCTAACCATATTTGCCTTGAG
Sef_Operon_F_EcoRI	AGGAATTCAGGAAACAGACCATGCGTAAATCAGCATC
SefD_R4_SacI	GCTCTAGAATGCCGTAGGAATGTCAAGC
Sta_Operon_F_SacI	CGGAGCTCAGGAAACAGACCATGAAAAAAGCGATTTTAGC

Sta_Operon_R_XbaI	ACTCTAGAATTGCGTTGCGGTTCGTT
Stb_Operon_F_SacI	CGGAGCTCAGGAAACAGACATGTCTATGAAAAAATATTTA
Stb_Operon_R_XbaI	GCTCTAGATGCTGAATTCTGGCCTGTC
Stc_Operon_F_XbaI	GCTCTAGAAGGAAACAGACCATGAAACGTTCACTTATT
Stc_Operon_R_SalI	CGGTCGACAGATTGTCATCCCGGTCACT
Std_Operon_F4_SacI	CGAGCTCATGCGTAATAAAATAATACTTGCC
Std_Operon_R3_XbaI	GCTCTAGAATTAGTTCCCCGATAACTCAGTCA
Ste_Operon_F_SacI	GCGAGCTCAGGAAACAGACCATGAAGTCATCTCATTTTTG
Tcf_Operon_F_SacI	CGCGAGCTCAAGAAACAGACCATGAATTTTAAAGATACTCTTCC
Tcf_Operon_R_XbaI	GCTCTAGAAAAAACCATATAAGAAAGATATCAA
Sth_Prom_F_BamHI	CGGGATCCAAATCCAGTCATCTACCGTACTTC
Fim_Prom_F_Short_BamHI	CGGGATCCGGCGGCATAATGCGACATTT
Saf-FC	CCCTCGAGGGAAGATAAGTTTCCCACACCC
Stc_Prom_F_XbaI	GCTCTAGAAATTCCGCAGGCCCATATCA
Std_Prom_F_SacI	CGAGCTCGTTCTTTGCTGTCGGCATTG
Ste_Prom_F_2_BamHI	CGGGATCCACAGGTGTTGAATGCTACCTTTCCC

*Restriction enzyme sites are underlined. Letter in italics represent overlapping sequences.

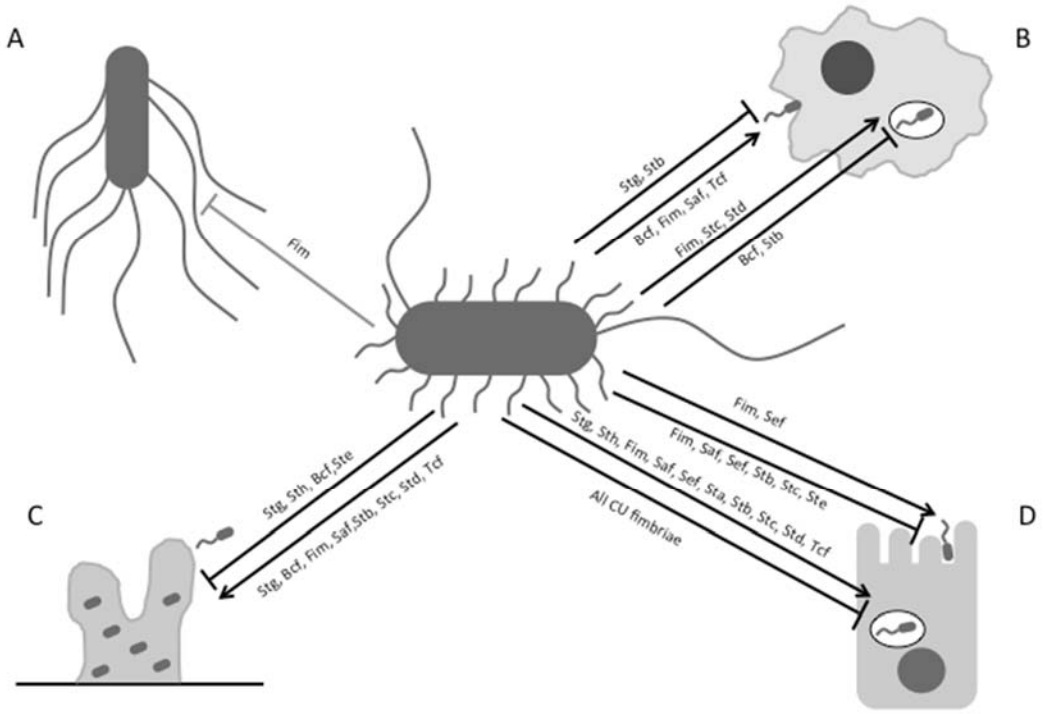

Figure 12. – Impact of *S.* Typhi fimbriae on motility, host cell interactions and biofilm formation.

A summary of the effects of each fimbrial gene clusters of *S*. Typhi is presented. (A) Effects on motility. (B) Effects on macrophages-bacteria interactions. (C) Effects on biofilm. (D) Effects on epithelial cells-bacteria interactions.

Chapitre 3 – Régulation du fimbria Std chez *Salmonella enterica* sérovar Typhi

3.1 Préface au chapitre

Le fimbria Std a été étudié chez *S*. Typhimurium et son implication dans la persistence intestinal chez un modèle murin a été établie. Il semble que Std s'attache aux résidus fucosylés présents à la surface des cellules épithéliales présentes dans le colon. Plusieurs régulateurs chez *S.* Typhimurium ont été identifiés comme Dam, HdfR, RosE, SeqA, StdE et StdF. Dans le prochain article, nous présentons l'étude de régulation que nous avons effectué chez *S*. Typhi pour confirmer l'effet des régulateurs connus chez *S.* Typhimurium, mais aussi afin d'identifier de nouveaux régulateurs et de mieux comprendre la fonction de ce fimbria chez *S*. Typhi. Cet article sera soumis à un processus de révision par les pairs probablement dans ''Journal of Bacteriology''.

Article 3 : Dufresne, K. et Daigle, F. 2020. Identification of Crp as a novel regulator of the Std fimbrial expression in *Salmonella*

Contribution des auteurs :

J'ai réalisé l'élaboration du projet, les différentes expériences, l'écriture du manuscrit et la création des figures et tableaux. France Daigle m'a conseillé tout au long du processus et a fourni le support financier au projet.

3.2 Article 3 – Identification of Crp as a novel regulator of the Std fimbrial expression in *Salmonella*

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Running title: Expression of Std fimbriae in *S*. Typhi

3.2.1 Abstract

Salmonella enterica Typhi genome contains 14 putative fimbrial systems. Std belongs to the chaperone-usher family and several regulators of Std were previously identified in *S.* Typhimurium, such as RosE, Dam, HdfR, SeqA and StdEF. However, *std* regulation has not been investigated in *S.* Typhi. We hypothesize that regulators of *S*. Typhimurium maybe shared with *S.* Typhi, but that several other regulators are to be discovered. Here, we describe the role of more than 50 different candidate regulators on *std* expression. Three types of regulators were investigated: known regulators in *S.* Typhimurium, *in silico* predicted regulators and virulence/metabolic regulators. *std* expression was determined in the regulators mutants and compared with the wild-type strain. Overall, 21 mutants affect *std* promoter expression. The role of Crp, a newly identified factor for *std* expression, was further investigated. Crp acted as activator of *std* expression on a distal region of the *std* promoter region. Altogether, our results demonstrated the major influence of Crp as a novel transcriptional factor on *std* promoter expression in *Salmonella*.

3.2.2 Importance

Std fimbriae were previously studied in *S.* Typhimurium but not in *S.* Typhi. The *std* operon encodes a chaperone-usher fimbria and two regulators (StdEF) that influence a large regulon including virulence factors. Std regulation is critical for optimal virulence in *Salmonella*. Here, we identified 21 modulators of *std* expression including Crp as a novel factor controlling the expression of the Std fimbria. Altogether, the influence of Crp on *std* activation is major and suggests that availability of different carbon sources is important for *std* expression in *Salmonella*.

3.2.3 Introduction

Salmonella enterica serovar Typhi (*S*. Typhi), the etiologic agent of typhoid fever, possesses a unique combination of 14 fimbriae. This repertoire includes one curli fimbria (Csg), one type IVb pilus (Pil) and 12 chaperone-usher fimbriae (Bcf, Fim, Saf, Sef Sta, Stb, Stc, Std, Ste, Stg, Sth, and

Tcf). Fimbriae are organized in cluster that encoded the structural subunits, proteins for the secretion, fimbrial assembly and regulatory proteins. Regarding the chaperone-usher fimbriae assembly, the external proteinaceous structural units are secreted in the periplasm where a dedicated chaperone will protect and export them across the periplasm to be delivered to the dedicated usher for final fimbria assembly [1].

Std fimbria is highly present in *Salmonella enterica,* except in subspecies *arizonae* and *salamae* [2]. This chaperone-usher fimbria belongs to the π fimbriae class, with an usher related to the P-like fimbriae [1]. The Std operon encoded a major subunit (StdA), an usher (StdB), a chaperone (StdC), an adhesin (StdD) and 2 regulator genes (StdEF) [3]. In *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), Std was associated with intestinal persistence in mice by specific binding of fucosylated residues [4, 5].

The *std* fimbrial operon is unusual because its operon encodes not only the structural genes but also regulators. Its expression needs to be tightly regulated as the two regulators, StdEF, can modulate SPI-1 genes, flagella and other virulence factors [3, 6, 7]. *std* expression was found to be modulated by several regulators, including the Dam methylation system, SeqA, HdfR, RosE and StdEF [3, 7, 8]. Dam acts as an inhibitor of *std* by binding of the second and third of the three GATC sites in the promoter region of the *std* operon and methylation of this site [7]. SeqA binds to the methylated GATC region [8]. HdfR is a transcriptional repressor of FlhDC in *Escherichia coli* (*E. coli*) by binding the upstream region of the gene by its helix-turn-helix LysR-type motif [8]. HdfR is repressed by H-NS and acts as an activator of *std* expression in a *dam* or *seqA* mutant strain [8]. HdfR was recently proposed to bind to a region in the *std* promoter near the GATC sites and to form an activating loop with StdE and StdF [7]. RosE was identified as an inhibitor of *std* expression [9]. The two regulators StdE and StdF were involved in the expression of *std* operon itself, but also in the regulation of a variety of virulence genes [3, 7]. The expression of these factors and the rest of the *std* operon is bistable, allowing the population for a division of bacteria between the Std^{ON} and Std^{OFF} state [7].

In *S.* Typhi, the regulation of fimbrial systems is poorly understood. We previously demonstrated that *std* was one of the highest expressed fimbriae among the 14 fimbriae of *S.* Typhi and was highly expressed when grown in minimal medium [2]. Interestingly, in a study comparing fimbrial degradation, *std* and *tcf* were the only intact fimbrial systems in all human-restricted strains [10]. As Std is conserved in both *S*. Typhi and *S.* Typhimurium, we hypothesis that some regulatory mechanisms will be shared and that novel regulators are to be discover. To determine if new mechanisms surrounding *S.* Typhi *std* expression and regulation could be unveil, we determine the role of known *std* regulators, the role of putative regulators identified *in silico* and the role of virulence modulators in *S.* Typhi. Each of these candidates was deleted from *S.* Typhi ISP1820 and the expression of *std* was assessed. We identified several regulators of *std*, including the cAMP receptor protein, Crp, as a novel factor for *std* expression, and we confirm the importance of HdfR and Dam on *std* regulation in *S.* Typhi.

3.2.4 Materials and methods

3.2.4.1 Bacteria, plasmids and growth conditions

Strains and vectors tested in this study are listed in Tableau 8. Bacteria were routinely grown on LB (Luria-Bertani) agar and cultured in LB broth. Experiments were performed in LB broth or in M63 [11]. When needed, antibiotics or supplements were used at the following concentrations: 34 ug/ml chloramphenicol, 100 ug/ml ampicillin, 50 ug/ml diaminopimelic acid (DAP). Introduction of plasmid by transformation was performed by calcium/manganese-based (CCMB) transformation or by electroporation [12].

3.2.4.2 Cloning of *stdA* promoters

The fusion of *stdA* promoter to *lacZ* gene on pRS415 vector is described in Dufresne, Saulnier-Bellemare [2]. We cloned also truncated versions of the *stdA* promoter to identify binding site of putative regulators: primers used for these cloning are listed in Tableau 9.

3.2.4.3 Role of putative regulators by chromosomal deletion

Known regulators of *std* in *S.* Typhimurium (HdfR, Dam, SeqA, RosE, StdE), putative regulators of *std* identified using BPROM (Softberry), (Ada, ArgR, Fur, Lrp, NagC, PhoB, SoxRS) and regulators of virulence such as FlhCD, InvA, and PhoP were deleted. The exhaustive list of all mutants tested is presented in Tableau 7. Mutant strains were constructed by allelic exchange mutagenesis as described in Forest, Faucher [13]. Mutant strains were confirmed by PCR and Sanger sequencing (data not shown). Mutant strains and primers used for deletion are listed in Table S1 and Table S2 respectively.

3.2.4.4 β-galactosidase activity assays

Expression of the *stdA* promoter in the different strains was assessed by β-galactosidase assay as described in Miller [14]. After an overnight growth with agitations in LB broth or in M63 minimal broth, the cells were lysed and the substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG) was added. The optical density for 420 and 550 nm was assessed and Miller units were calculated. The results are presented as the mean ± SEM of the expression ratio where the Miller units of each studied strains were compared to the associated WT strain. The data are representative of at least 3 independent experiments done in duplicate.

3.2.4.5 Western Blot

After overnight culture in M63 broth, fimbriae were harvested after a thermal shock (60 °C) and vortexing. The culture was centrifuged and the resulting supernatant containing the extracellular structures was precipitated by trichloroacetic acid (TCA) and then loaded on SDS-PAGE (15%). Proteins were transferred on PVDF membrane and blocked by TBST 5% skimmed milk. The membrane was incubated with polyclonal rabbit anti-StdA (kindly provided by A.J. Baumler) and then to HRP-conjugated anti-rabbit secondary antibody. Finally, the membrane was incubated with Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare). The membrane was analyzed with the Amersham Imager 600 (GE Healthcare). The results presented are representative of at least 3 independent experiments.

3.2.4.6 qPCR assay

Total mRNA was extracted after an overnight culture in M63 broth. Briefly, bacteria were suspended in Trizol (Invitrogen) as recommended by the manufacturer. The RNA samples were treated with Turbo DNAse (Ambion). Complementary DNA (cDNA) was synthesized using the Superscript II reverse transcriptase (Invitrogen). Quantitative PCR (qPCR) on *stdA* and 16S ribosomal RNA was performed using the Quanta SyBR Green PCR Master Mix. Fold change of *stdA* was normalized against 16S rRNA. The experiments were performed at least 3 independent times.

3.2.4.7 Statistical Analysis

Statistical analysis was performed on GraphPad Prism. Two-tailed unpaired Student's *t*-test was applied on data sets for each construction compared to their control data. $P < 0.05$ was considered significant.

3.2.5 Results

3.2.5.1 Identification of novel regulators for *S*. Typhi *std* operon

To identify regulators of *S.* Typhi *std* fimbriae, *stdA* expression was evaluated in 5 mutants of known regulators of *std* in *S.* Typhimurium (RosE, HdfR, Dam, SeqA and StdE), in 7 mutants of predicted regulators of the *S.* Typhi *stdA* promoter region and in 41 mutants of regulators of global virulence or metabolism (Tableau 7). Regulators were deleted from the wild-type strain ISP1820 that was used as control for the *std* promoter expression.

Tableau 7. – List of all putative regulators tested for *std* promoter expression

Type of regulator	Gene name
Predicted regulators (BPROM)	*ada, argR, fur, lrp, nagC, phoB, soxRS*
S. Typhimurium known regulators	*dam, hdfR, rosE, seqA, stdE*
Virulence and metabolic regulators	*arcA, assT, baeR, barA, bacfABCDEFG, cdtB, cheY, citB, cpxR, creB, crp, csgCABDEFG, dpiA, feoAB, fimAICDHFZYXW, flhCD, glnG, hilD, hydG, katG, kdpE, leuO, lrhA, narL, narP, ompRenvZ, oxyR, pflA, phoP, qseF, rcsDBC, rpoN, rstA, sirA, sodB, SPI-1 (invA), SPI-2 (ssaU-ssrB), staABCDEFG, tviA, ydiV, ygaA*

Overall, we tested 53 different mutants (Tableau 7) including mutant of the type III secretion systems (T3SS) on the *Salmonella* pathogenicity islands (SPI) -1 and -2, capsule, typhoid toxin, flagella and metabolic pathway genes. There were 21 mutants that showed a significant difference ($p \leq 0,05$) of the *stdA* expression compared to the wild-type strain (Figure 13).

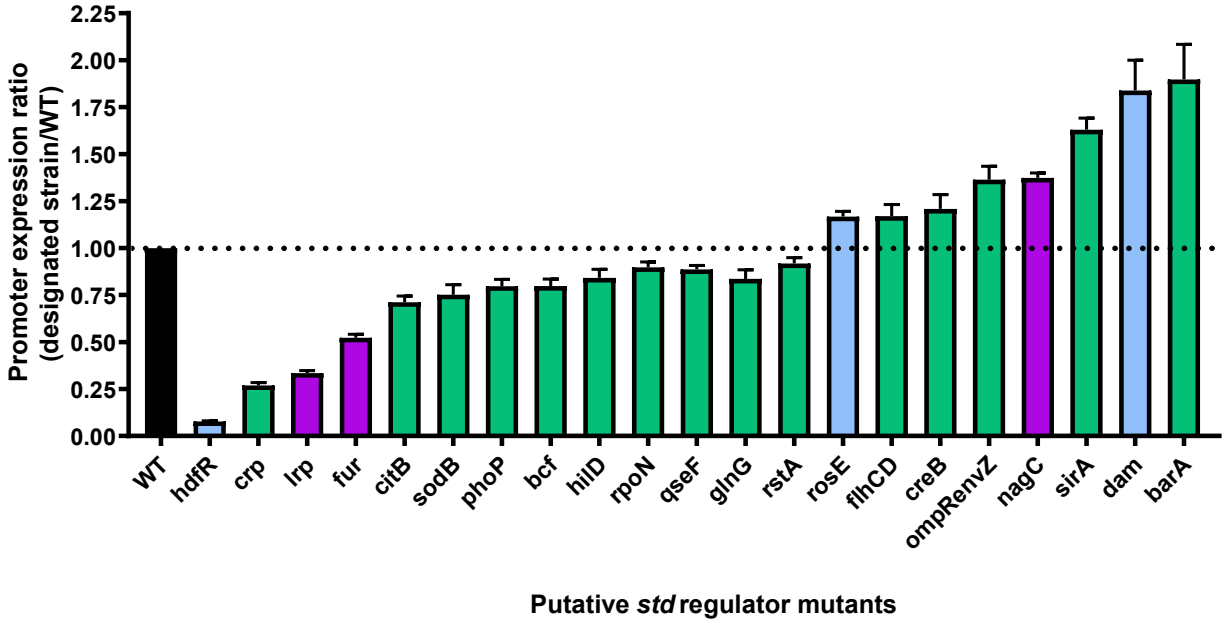

Figure 13. – Significant modulators of *std* promoter expression in *S.* Typhi ISP1820.

Putative modulators of *std* expression were tested for *std* promoter expression in LB broth and are classified in three categories: known regulators in *S.* Typhimurium (blue bars), predicted regulators by BPROM (purple bars) and metabolic/virulence regulators (green bars). All the regulators were compared for *std* promoter to WT strain (black bar) expression by β-galactosidase assay. The results are presented as the ratio of expression between each strain and their respective WT Miller units. The mean of the ratio is showed in the figure ± SEM and is representative of 6 replicates at least. Only the putative regulators that demonstrated significant difference ($p < 0,05$) are showed.

The majority of *std* regulators known in *S.* Typhimurium (HdfR, RosE, Dam) had a similar effect on the *std* expression in *S.* Typhi. However, the *stdE* and *seqA* mutants shown no significant difference in *S.* Typhi (data not shown). From *in silico* prediction, the regulators Fur, Lrp and NagC have significant difference for *std* expression. Regarding the global virulence regulators, HilD, one of the regulators of SPI-1, has a minor activator effect on *std* expression, but InvA had no significant difference. Several two-component systems (TCS) showed modulation of *std* promoter: PhoP, QseF, GlnG and RstA as activator of *std* and CreB, OmpR/EnvZ and BarA as inhibitor. We have also identified metabolic regulators such as Crp, CitB, SodB and RpoN involved in *std* expression and finally the major flagella regulator FlhCD and the Bcf fimbria acted as regulators of the *std* expression.

3.2.5.2 Crp activates *std* expression in *S*. Typhi

We further confirmed the role of a novel identified regulator Crp. Dam and HdfR, two major known regulators of *S*. Typhimurium *std* operon, were used as controls as they bind in the promoter region of *stdA* in *S.* Typhimurium. In addition to the single mutant of each of these 3 regulators, we constructed double mutants of Crp and Dam (Δ*CD*) and Crp and HdfR (Δ*HD*). The bacteria were grown in M63 broth to promote high expression of *std* promoter as demonstrated in Dufresne et *al* [2]. Single deletion of *crp* or *hdfR* decreased the expression of *std* in M63 and the *dam* mutant increased expression (Figure 14A). The double mutant Δ*CD* has a higher expression than *Δdam* mutant and Δ*HD* has a *std* expression similar to the single deletion of *hdfR*.

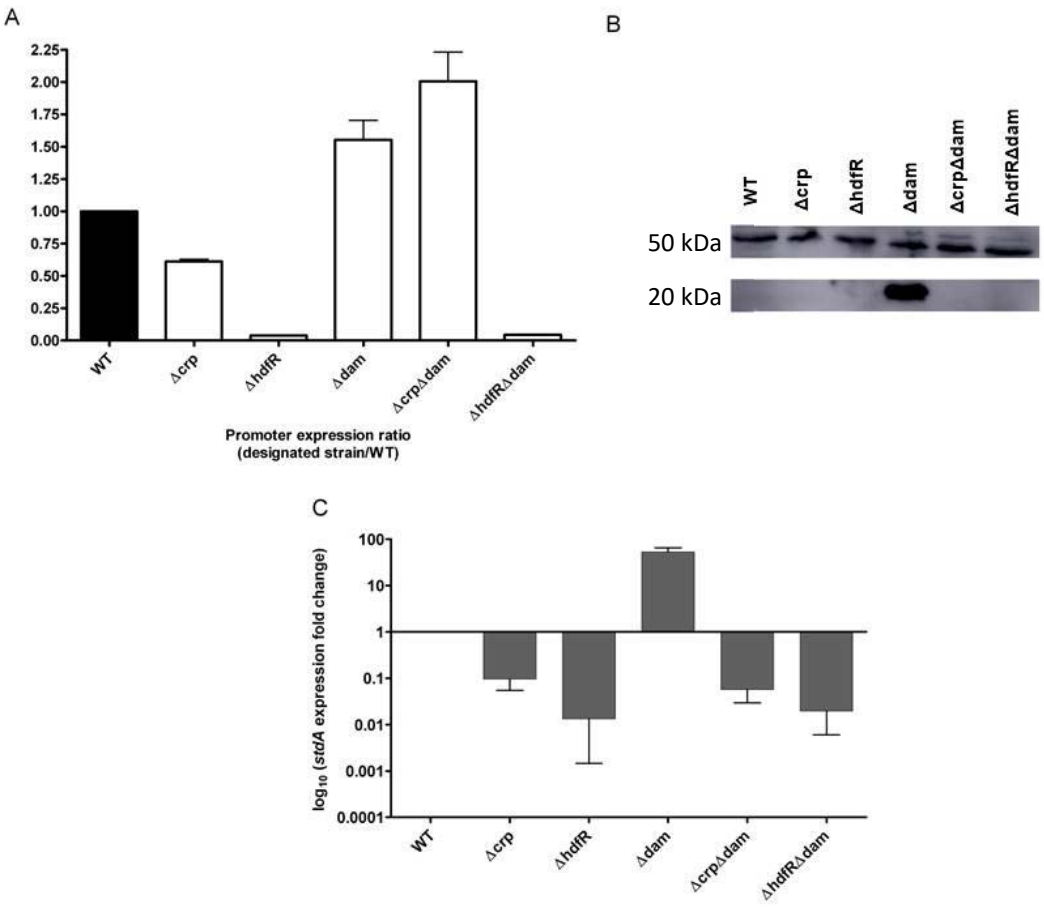

Figure 14. – Crp is an activator of *std* expression.

Single and double mutant strain for *crp* and two controls (*hdfR* and *dam*) were tested for *std* promoter expression, StdA production and mRNA expression in M63 broth. (A) The results of the β-galactosidase assay are presented as the ratio of expression between each strain and their respective WT Miller units. Results are presented as the mean ± SEM of replicas. (B) The Western blot specific to StdA protein is representative of at least three independent experiments. (C) The results of mRNA expression are presented as the logarithmic fold change compared to the WT strain. Results are presented as the mean ± SEM of replicas.

3.2.5.3 Production of Std fimbriae and mRNA expression

To confirm the effects of Crp on Std fimbria production, detection of the major subunit StdA by Western Blot was evaluated in M63 broth. Only the *S.* Typhi Δ*dam* strain allows the production of enough StdA to be vizualized by Western Blot (Figure 14B). We did not observe StdA either in the double mutants Δ*CD* and Δ*HD*. To understand the difference between the promoter expression results and the StdA production of the Δ*crp*Δ*dam* mutant, a RT-qPCR was performed (Figure 14C). It shows that Δ*dam* has the highest *stdA* mRNA expression followed by the WT strain and that the other tested strains (Δ*crp,* Δ*hdfR,* Δ*crp*Δ*dam* and Δ*hdfR*Δ*dam*) had weaker mRNA than the WT. The mutant Δ*crp*Δ*dam* has similar expression to Δ*crp* and Δ*hdfR*Δ*dam* to Δ*hdfR*.

3.2.5.4 Crp binds to a distal region of the *std* promoter

In order to confirm that Crp acts directly on the *std* promoter, we created 3 truncated version of the *std* promoter-*lacZ* fusion (Figure 15A). The entire *std* promoter is 713 bp. The pRS415*std*-C is a 619 bp fragment that deletes a putative Crp-binding motif (TCTGA-N6-TCACA). The pRS415*std*-CL is a 480 bp that deletes the region that includes Crp, Lrp and Fur putative binding sites. The pRS415*std*-CLD is a 239 bp that deleted also the 3 GATC sites, corresponding to the binding site of Dam and HdfR proteins [7]. These reporters were transformed in the WT and Δ*crp* strains.

In the WT strain, each truncated version of pRS415*std* (-C, -CL and –CLD) cause a major decrease in *std* expression (Figure 15B). The mutant strain Δ*crp* has similar *std* promoter expression level than the wild-type strain for truncated version of pRS415*std* (pRS415*std*-C, -CL and -CLD).

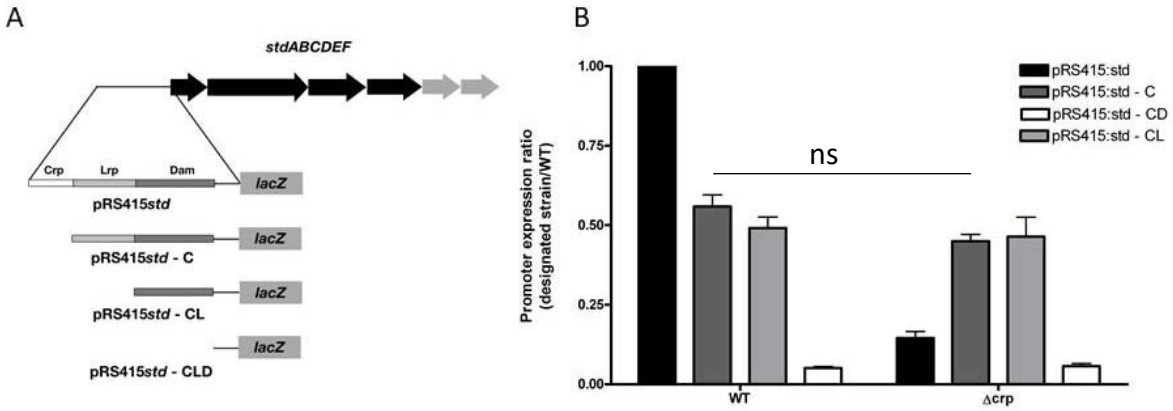

Figure 15. – Truncation of *std* promoter region affects modulation by Crp.

(A) Truncation of the *std* (pRS415*std*) in three shorter versions (pRS415*std*-C, -CL and –CLD) and transformed into WT and *Δcrp* strains. (B) The results of the β-galactosidase assay are presents as the ratio of expression between each strain and their respective WT Miller units. The experiment was performed at least three times independently and is presented as the mean of results ± SEM.

3.2.6 Discussion

The *std* operon was one of the most expressed fimbriae of *S.* Typhi [2]. We aimed to identify regulators that explain this high expression. The approach used in this study targets candidate regulators from known, predicted and general regulators of virulence or metabolic pathway. Each gene of interest was mutated by allelic exchange and transformed with the vector pRS415*std* to assess the *stdA* promoter expression. We tested 53 different mutants and overall 21 mutants demonstrated significant modulation of *std* expression (Figure 13). We confirmed similar modulation of *std* expression for Dam, RosE and HdfR than in *S.* Typhimurium, but SeqA and StdE have no significant effect in *S.* Typhi. We also identified novel modulators of *std* expression: Fur, Lrp, NagC, PhoP, QseF, GlnG, RstA, CreB, OmpR/EnvZ, SirA, BarA, HilD, Crp, CitB, SodB, RpoN, Bcf and FlhCD. These regulators and their underlying mechanisms need to be further study to determine if they act directly or indirectly on *std*. Interestingly, many regulators associated with nutrient limitations responses and envelope perturbations were involved in *std* expression.

Among these mutants, we pursue to study Crp as a major difference of expression in comparison with the WT strain was observed. We have also demonstrated that *std* expression was affected in a *crp* mutant in *S.* Typhimurium SL1344 (Figure 16). As a lower level of *std* expression was observed in the Δ*crp* strain, we evaluated if the Crp regulation was at the transcriptional or translational level (Figure 14). The deletion of *crp* decrease *std* mRNA expression which results in less StdA in the extracellular proteinaceous extract. Crp is a novel regulator of *std* identified in this study. Crp was originally identified for its role in catabolic repression and in carbohydrates metabolism. Its regulation role was extended to many virulence factors in several different bacteria species [15-18]. In *S.* Typhi, Crp was identified as regulator of *hlyE* and *taiA*, virulence genes encoded on SPI-18 [15, 17]. Regarding fimbrial regulation, Crp was identified as regulator of fimbriae in *Serratia marcescens* and *Klebsiella pneumoniae* [18-20]. It was also identified in uropathogenic *E. coli* as an activator of the Pap fimbriae, genetically similar fimbriae to Std [1, 18, 21].

Overall, we confirm that Crp acts as a direct activator of *std* operon in *S.* Typhi at the distal region of the promoter region. Carbon metabolism and carbohydrates utilization are key elements for survival of living organisms. It is principally regulated by Crp and acts as a signal for virulence. Crp will be activated or inhibited in presence of different carbon sources that will modulate its regulon [19]. By example, in presence of increasing level of glucose, Crp is inactivated and *std* expression decreases (Figure 17).

In this study, we screened several regulators involved in *std* expression. We confirmed that HdfR, RosE and Dam are also modulator of *std* in *S*. Typhi like it was previously demonstrated in *S*. Typhimurium. Overall, we identified 21 regulators that significantly modulate *std* expression, including 18 new ones. Crp is as a novel activator of *std* acting directly at the promoter region. Carbohydrates are key nutrients for bacteria and for their host: limitation or change in carbon source can trigger virulence factors like fimbriae. It seems to be the case for *S.* Typhi Crp that activate expression and production of Std fimbriae. Altogether, these data propose that *std* is expressed in *S.* Typhi by sensing the presence of different carbon sources. The role of Crp on Std fimbria should be further studied to better understand the importance of Std for virulence in *S.* Typhi and other *Salmonella enterica* serovars. Also, it would be interesting to determine if Std had the same function than in *S.* Typhimurium at the intestinal level.

3.2.7 References

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3.2.8 Supplementary data

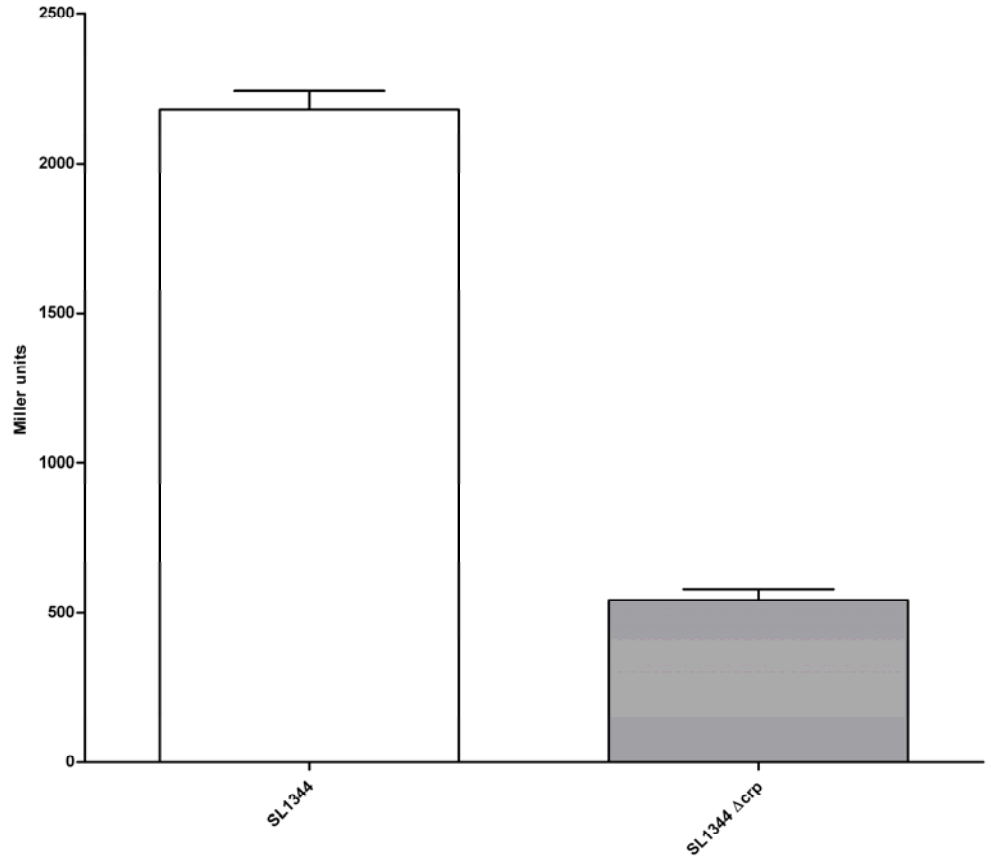

Figure 16. – Crp activates std promoter in *S.* Typhimurium.

WT and *Δcrp* strains of SL1344 were transformed with pRS415*std* and β-galactosidase assay was performed. Results are presented as the mean ± SEM of Miller units for each strain and are representatives of at least 6 replicates.

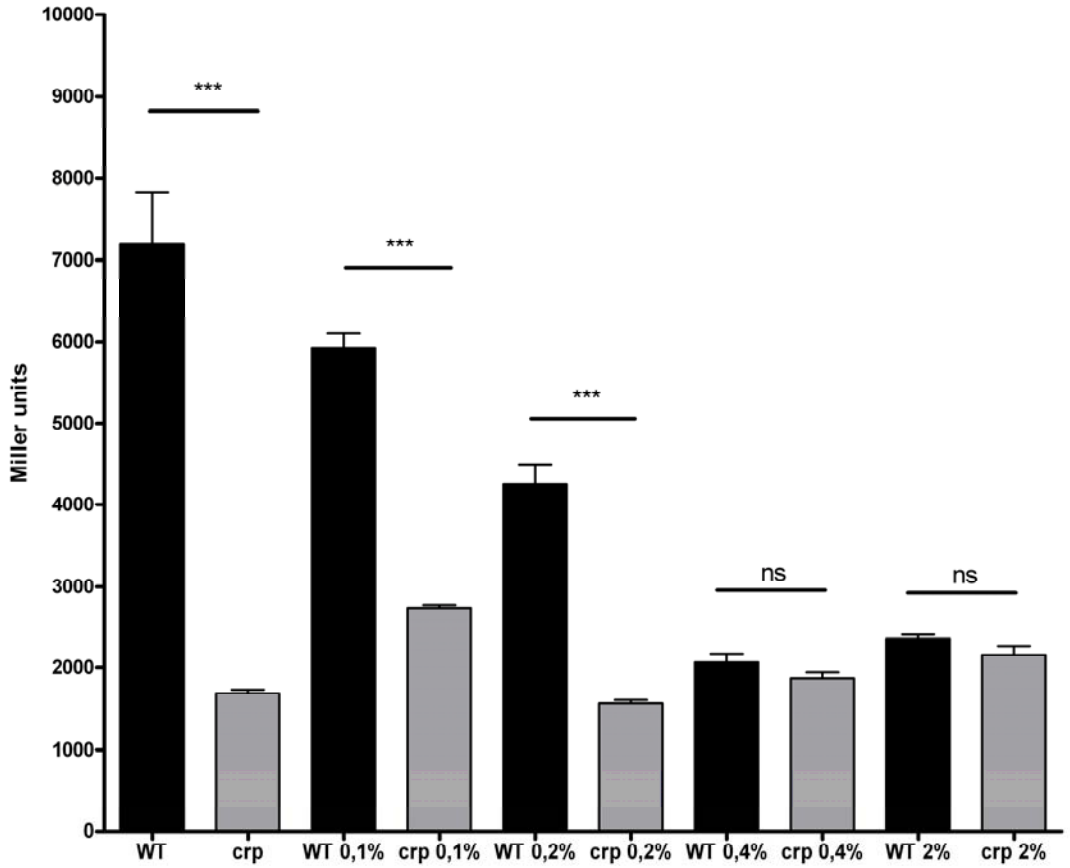

Figure 17. – Effect of glucose concentration on *std* expression in WT and *crp* strain.

WT/pRS415std (black bars) and *Δcrp*/pRS415std (grey bars) were incubated in LB broth with or without different concentrations of glucose (between 0 and 2% glucose). Results are presented as the mean ± SEM of Miller units for each strain and are representatives of at least 3 replicates.

Tableau 8. – Bacterial strains and plasmids used for this study

Strain or plasmid	Name	Characteristic	Vector used for deletion	Source or reference
		***S.* Typhi**		
DEF1045	WT	ISP1820 wild-type		R. Curtiss III, U. Florida
DEF1355	*ΔhdfR*	ISP1820 *ΔhdfR*	pSIF524	This study
DEF1342	*Δcrp*	ISP1820 *Δcrp*	pSIF504	This study
DEF1359	*Δdam*	ISP1820 *Δdam*	pSIF527	This study
DEF1567	*ΔDC*	ISP1820 *ΔdamΔcrp*	pSIF527/ pSIF504	This study
DEF1587	*ΔHD*	ISP1820 *ΔhdfRΔdam*	pSIF524/ pSIF527	This study
DEF1540	*ΔhilD*	ISP1820 *ΔhilD*	pSIF563	This study
DEF1361	*ΔstdE*	ISP1820 *ΔstdE*	pSIF529	This study
DEF1568	*ΔrosE*	ISP1820 *ΔrosE*	pSIF528	This study
DEF571	*ΔflhCD*	ISP1820 *ΔflhCD*	pSIF184	(Sabbagh et al, 2012)
DEF490	*Δfur*	ISP1820 *Δfur*	pSIF130	(Leclerc et al, 2013)
DEF1323	*ΔcitB*	ISP1820 *ΔcitB*	pSIF514	(Murret-Labarthe et al, 2019)
DEF1241	*ΔphoP*	ISP1820 *ΔphoP*	pSIF478	(Murret-Labarthe et al, 2019)
DEF1307	*ΔglnG*	ISP1820 *ΔglnG*	pSIF485	(Murret-Labarthe et al, 2019)
DEF1512	*ΔrpoN*	ISP1820 *ΔrpoN*	pSIF551	This study

DEF1329	*Δlrp*	ISP1820 *Δlrp*	pSIF503	This study
DEF1310	*ΔqseF*	ISP1820 *ΔqseF*	pSIF497	(Murret-Labarthe et al, 2019)
DEF1336	*ΔnagC*	ISP1820 *ΔnagC*	pSIF501	This study
DEF1311	*ΔrstA*	ISP1820 *ΔrstA*	pSIF498	(Murret-Labarthe et al, 2019)
DEF569	*ΔbarA*	ISP1820 *ΔbarA*	pSIF185	(Murret-Labarthe et al, 2019)
DEF1435	*ΔsodB*	ISP1820 *ΔsodB*	pSIF540	(Murret-Labarthe et al, 2019)
DEF1254	*ΔcreB*	ISP1820 *ΔcreB*	pSIF483	(Murret-Labarthe et al, 2019)
DEF863	*ΔompRΔenvZ*	ISP1820 *ΔompRenvZ*	pSIF283	(Murret-Labarthe et al, 2019)
DES1247	*ΔsirA*	ISP1820 *ΔsirA*	pSIF479	(Murret-Labarthe et al, 2019)
DEF563	*Δbcf*	ISP1820 *ΔbcfABCDEFGH*	pSIF175	(Sabbagh et al, 2012)
DEF1358	*Δada*	ISP1820 *Δada*	pSIF526	This study
DEF875	*ΔargR*	ISP1820 *ΔargR*	pSIF352	This study
DEF570	*ΔphoB*	ISP1820 *ΔphoB*	pSIF182	This study
DEF884	*ΔsoxRS*	ISP1820 *ΔsoxRS*	pSIF280	This study
DEF1239	*ΔarcA*	ISP1820 *ΔarcA*	pSIF475	(Murret-Labarthe et al, 2019)
DEF1513	*ΔassT*	ISP1820 *ΔassT*	pSIF547	This study

DEF1248	*ΔbaeR*	ISP1820 *ΔbaeR*	pSIF480	(Murret-Labarthe et al, 2019)
DEF1429	*ΔcdtB*	ISP1820 *ΔcdtB*	pSIF537	This study
DEF1522	*ΔcheY*	ISP1820 *ΔcheY*	pSIF553	This study
DEF1238	*ΔcpxR*	ISP1820 *ΔcpxR*	pSIF476	(Murret-Labarthe et al, 2019)
DEF510	*Δcsg*	ISP1820 *ΔcsgCABDEFG*	pSIF140	(Sabbagh et al, 2012)
DEF1253	*ΔdpiA*	ISP1820 *ΔdpiA*	pSIF482	(Murret-Labarthe et al, 2019)
DEF1056	*ΔfeoAB*	ISP1820 *ΔfeoAB*	pSIF418	This study
	Δfim	ISP1820 *ΔfimAICDHFZYXW*	pSIF064	(Dufresne et al, 2018)
DEF1318	*ΔhydG*	ISP1820 *ΔhydG*	pSIF509	(Murret-Labarthe et al, 2019)
DEF1509	*ΔkatG*	ISP1820 *ΔkatG*	pSIF550	This study
DEF1240	*ΔkdpE*	ISP1820 *ΔkdpE*	pSIF477	(Murret-Labarthe et al, 2019)
DEF760	*ΔleuO*	ISP1820 *ΔleuO*	pSIF269	(Sabbagh et al, 2012)
DEF432	*ΔlrhA*	ISP1820 *ΔlrhA*	pSIF107	This study
DEF1289	*ΔnarL*	ISP1820 *ΔnarL*	pSIF486	(Murret-Labarthe et al, 2019)
DEF1290	*ΔnarP*	ISP1820 *ΔnarP*	pSIF487	(Murret-Labarthe et al, 2019)
DEF1526	*ΔoxyR*	ISP1820 *ΔoxyR*	pSIF535	This study
DEF809	*ΔpflA*	ISP1820 *ΔpflA*	pSIF330	This study

DEF524	*ΔrcsDBC*	ISP1820 *ΔrcsDBC*	pSIF148	This study
DEF147	*ΔSPI-1*	ISP1820 *ΔinvA*	pSIF072	(Faucher et al, 2009)
DEF574	*ΔSPI-2*	ISP1820 *ΔssaU-ssrB*	pSIF187	(Forest et al, 2010)
DEF044	*Δsta*	ISP1820 *ΔstaABCDEFG*	pSIF046	(Dufresne et al, 2018)
DEF434	*ΔtviA*	ISP1820 *ΔtviA*	pSIF112	This study
DEF1565	*ΔydiV*	ISP1820 *ΔydiV*	pSIF571	This study
DEF1515	*ΔygaA*	ISP1820 *ΔygaA*	pSIF552	This study
DEF1365	*ΔseqA*	ISP1820 *ΔseqA*	pSIF531	This study
DEF1153	pRS	WT (pRS415)		(Dufresne et al, 2018)
DEF992	pRS*std*	WT (pSIF467)		(Dufresne et al, 2018)
DEF1356	*ΔhdfR*/ pRS*std*	*ΔhdfR* (pSIF467)		This study
DEF1507	*Δcrp*/pRS*std*	*Δcrp* (pSIF467)		This study
DEF1377	*Δdam*/pRS*std*	*Δdam* (pSIF467)		This study
DEF1578	*ΔDC*/pRS*std*	*ΔDC* (pSIF467)		This study
DEF1594	*ΔHD*/pRS*std*	*ΔHD* (pSIF467)		This study
DEF1541	*ΔhilD*/pRS*std*	*ΔhilD* (pSIF467)		This study
DEF1379	*ΔstdE/*pRS*std*	*ΔstdE* (pSIF467)		This study
DEF1579	*ΔrosE/*pRS*std*	*ΔrosE* (pSIF467)		This study
DEF1211	*ΔflhCD/* pRS*std*	*ΔflhCD* (pSIF467)		This study
DEF1213	*Δfur/*pRS*std*	*Δfur* (pSIF467)		This study
DEF1440	*ΔcitB/*pRS*std*	*ΔcitB* (pSIF467)		This study

DEF1293	*ΔphoP/* pRS*std*	*ΔphoP* (pSIF467)	This study
DEF1465	*ΔglnG/*pRS*std*	*ΔglnG* (pSIF467)	This study
DEF1517	*ΔrpoN/* pRS*std*	*ΔrpoN* (pSIF467)	This study
DEF1497	*Δlrp/*pRS*std*	*Δlrp* (pSIF467)	This study
DEF1500	*ΔqseF/*pRS*std*	*ΔqseF* (pSIF467)	This study
DEF1506	*ΔnagC/* pRS*std*	*ΔnagC* (pSIF467)	This study
DEF1434	*ΔrstA/*pRS*std*	*ΔrstA* (pSIF467)	This study
DEF1605	*ΔbarA/* pRS*std*	*ΔbarA* (pSIF467)	This study
DEF1441	*ΔsodB/* pRS*std*	*ΔsodB* (pSIF467)	This study
DEF1295	*ΔcreB/*pRS*std*	*ΔcreB* (pSIF467)	This study
DEF1214	*ΔompRΔenvZ/* pRS*std*	*ΔompRΔenvZ* (pSIF467)	This study
DEF1291	*ΔsirA/*pRS*std*	*ΔsirA* (pSIF467)	This study
DEF1376	*Δada/*pRS*std*	*Δada* (pSIF467)	This study
DEF1255	*ΔargR/*pRS*std*	*ΔargR* (pSIF467)	This study
DEF1256	*ΔphoB/* pRS*std*	*ΔphoB* (pSIF467)	This study
DEF1209	*ΔsoxRS/* pRS*std*	*ΔsoxRS* (pSIF467)	This study
DEF1378	*ΔseqA/* pRS*std*	*ΔseqA* (pSIF467)	This study
DEF1258	*ΔarcA/*pRS*std*	*ΔarcA* (pSIF467)	This study
DEF1539	*ΔassT/*pRS*std*	*ΔassT* (pSIF467)	This study
DEF1259	*ΔbaeR/* pRS*std*	*ΔbaeR* (pSIF467)	This study

DEF1431	*ΔcdtB/*pRS*std*	*ΔcdtB* (pSIF467)	This study
DEF1632	*ΔcheY/*pRS*std*	*ΔcheY* (pSIF467)	This study
DEF1292	*ΔcpxR/* pRS*std*	*ΔcpxR* (pSIF467)	This study
DEF1250	*Δcsg/*pRS*std*	*Δcsg* (pSIF467)	This study
DEF1294	*ΔdpiA/*pRS*std*	*ΔdpiA* (pSIF467)	This study
DEF1464	*ΔfeoAB/* pRS*std*	*ΔfeoAB* (pSIF467)	This study
DEF1249	*Δfim/*pRS*std*	*Δfim* (pSIF467)	This study
DEF1499	*ΔhydG/* pRS*std*	*ΔhydG* (pSIF467)	This study
DEF1510	*ΔkatG/* pRS*std*	*ΔkatG* (pSIF467)	This study
DEF1257	*ΔkdpE/* pRS*std*	*ΔkdpE* (pSIF467)	This study
DEF1216	*ΔleuO/*pRS*std*	*ΔleuO* (pSIF467)	This study
DEF1212	*ΔlrhA/*pRS*std*	*ΔlrhA* (pSIF467)	This study
DEF1468	*ΔnarL/*pRS*std*	*ΔnarL* (pSIF467)	This study
DEF1469	*ΔnarP/* pRS*std*	*ΔnarP* (pSIF467)	This study
DEF1529	*ΔoxyR/* pRS*std*	*ΔoxyR* (pSIF467)	This study
DEF1606	*ΔpflA/*pRS*std*	*ΔpflA* (pSIF467)	This study
DEF1208	*ΔrcsDBC/* pRS*std*	*ΔrcsDBC* (pSIF467)	This study
DEF1251	*ΔSPI-1/* pRS*std*	*ΔSPI-1* (pSIF467)	This study
DEF1252	*ΔSPI-2/* pRS*std*	*ΔSPI-2* (pSIF467)	This study
DEF1467	*Δsta/*pRS*std*	*Δsta* (pSIF467)	This study
DEF1210	*ΔtviA/*pRS*std*	*ΔtviA* (pSIF467)	This study

DEF1577	*ΔydiV/*pRS*std*	*ΔydiV* (pSIF467)	This study
DEF1519	*ΔygaA/* pRS*std*	*ΔygaA* (pSIF467)	This study
DEF1466	*Δbcf/*pRS*std*	*ΔbcfABCDEFGH* (pSIF467)	This study
		***S.* Typhimurium**	
DEF1041	SL1344	SL1344 wild-type	(Gulig et Curtiss, 1987)
DEF1562	SL1344 *Δcrp*	SL1344 *Δcrp*	This study
DEF1575	*SL Δcrp/* pRS*std*	SL1344 *Δcrp* (pSIF467)	This study
		***E.* coli**	
DEF1162	MGN-617	SM10 λpir asd thi thr leu tonA lacY supE recA RP4 2-Tc : :Mu[λpir] *asdA4*	(Kaniga *et al*., 1998)
		Plasmids	
pMEG375		*sacRB mobRP4 oriR6K*, Cm^r Ap^r	R. Curtiss III, U. Florida
pRS415		Multicopy vector with a promotorless, *lacZ* reporter gene, Ap^r	(Simons *et al.*, 1987)
pSIF467	pRS415*std*	pRS415 carrying the promoter region of *stdA*	(Dufresne et al, 2018)
pSIF582	pRS415*std* -C	pRS415 carrying the promoter region of *stdA*	This study
pSIF581	pRS415*std* -CL	pRS415 carrying the promoter region of *stdA*	This study
pSIF580	pRS415*std*-CLD	pRS415 carrying the promoter region of *stdA*	This study

Tableau 9. – Primers used in this study

Primers	**Sequence (5'-3')***
Std_Prom_F_EcoRI	GCGAATTCGTTCTTTGCTGTCGGCATTG
Std_Prom_F-crp_EcoRI	CAGAATTCATACAGAAGAATATCATTTC
Std_Prom_F-crplrp_EcoRI	CAGAATTCCATTGTTATATATGATTAGTATTATGT
Std_Prom_F-crplrpdam_EcoRI	CAGAATTCCTTACAACTGATTAGGCATTAGC
Std_Prom_R_BamHI	AAGGATCCACAGAAGCGCCATACATCATACCG
hdfR F1	CGGGATCCTCCTGCACGCTCCTAATTCT
hdfR R2	*CATCCGTTT*CGGATTGCGTCAGGTAAAGT
hdfR F3	*CGCAATCCG*AAACGGATGTGCTGGATGAA
hdfR R4	GCTCTAGAGGGGCGTAGTTCAATTGGTA
dam F1	CGGGATCCCTGCAATTGCCTGTGAGTGT
dam R2	*CTCGCGTGT*ACCCACAAAAGGTTCGACAA
dam F3	*TTTGTGGGT*ACACGCGAGTGGTATCAAC
dam R4	GCTCTAGAATCAGCCGACAGAATTGAGG
crp F1	CGGGATCCTGCAACCTCAGAGACAGTG
crp R2	*CCCATCCGG*CACGGAGCCTTTAACGATGT
crp F3	*GGCTCCGTG*CCGGATGGGATGCAGATCA
crp R4	GCTCTAGAGCCACATCGATGCAAACAAA
hilD F1	CGGGATCCCCACCTGATACCTTAAGTTCG
hilD R2	*TCATTCGCG*CGGACGATGTCGTAATTGAA

hilD F3	*CATCGTCCG*CGCGAATGAATAAAGCGACAA
hilD R4	GCTCTAGATAGCGAGCAACAGAATTCCA
stdE F1	CGGGATCCCTGTTCTTCACCTCCGGAAA
stdE R2	*GCACAGCCG*CCAGTGTGCCACGATGATA
stdE F3	*GCACACTGG*CGGCTGTGCAGTAACAGAAA
stdE R4	GCTCTAGAATCAGTATGGCCTCCGGTTT
rosE F1	CGGGATCCATTACCGTCCTTCCCATTCC
rosE R2	*TCTCGCCCG*CGTTGCCATGCACTAACACT
rosE F3	*ATGGCAACG*CGGGCGAGAAAGGTACTTTGA
rosE R4	GCTCTAGACAGATCGATGAGGTGTTGGA
rpoN F1	CGGGATCCCCTGCAAGACGAACACGTTA
rpoN R2	*CTCAGTGGT*TCAAGCAGCGGGTTATTTTC
rpoN F3	*GCTGCTTGA*ACCACTGAGCGACAGCAAG
rpoN R4	GCTCTAGAGCAGGGCTTCAGTAATTTCG
lrp F1	CGGGATCCCCGGGCTAGTGAAATCTACG
lrp R2	*GGTGTCGTT*CCCATCCTTTTGCAGTTCAT
lrp F3	*AAGGATGGG*AACGACACCCGAACTTACG
lrp R4	GCTCTAGACTCTCCAGGTTCCAGGCTTT
nagC F1	CGGGATCCGGTGACGAGGTCGTTGACTT
nagC R2	*ACGGTGATC*AGAGGCCTGCTGATCGACT
nagC F3	*CAGGCCTCT*GATCACCGTTCTGCTATCG

nagC R4	GCTCTAGAGACGGGTAATTGGTCAGCA
ada F1	CGGGATCCCCGCAGTTCAAAACATTCCT
ada R2	*TAGCTGCGC*CCAGCATTCATCATCGGTAA
ada F3	*GAATGCTGG*GCGCAGCTATTAAAGCGAGAAG
ada R4	GCTCTAGATGACTGACTGGCGACAAATC
argR F	GCTCTAGAGCAACGTTCTACAGGTGGTG
argR Rover	*AAAAGCCGC*TAGCCGAGCTTCGCATAAGT
argR Fover	*GCTCGGCTA*GCGGCTTTTCCGTAAGAGAT
argR R	CGGGATCCCCGCTGTCGTTTGAAAAATAA
fur-F	CGGGATCCCGACATCCTCAACGCCTAA
fur-Rover	*GTCATCGTG*GCGGAATCTGTCCTGTTG
fur-Fover	*AGATTCCGC*CACGATGACGCGACTAAA
fur-R	GCTCTAGAGCCTGTGAATAAAAGGCCAG
PhoB F1	CGGGATCCCATGC TGTCAATGCCGCCTT
PhoB R2	*CGAATATGC*TCTACGGGCTGAAAGCCATT
PhoB F3	*GCCCGTAGA*GCATATTCGCCGTTTGCGTA
PhoB R4	AAGGAAAAAAGCGGCCGCACCAGCCTTTT CCATGACAG
soxRS F	CGGGATCCGCAGTATTGTCAGGGATGGC
soxRS over R	*AAGCAGCCG*ACTCCCAGCGATTACCGTCA
soxRS over F	*GCTGGGAGT*CGGCTGCTTGAAGATGATTA
soxRS R	GCTCTAGACAGTATCAACACAAACCGGA

seqA F1	CGGGATCCACTGGGCTGCAACCTCTAAG
seqA R2	*ATGCTGCAT*GTGGCGGAAAACTTCAACAT
seqA F3	*TTCCGCCAC*ATGCAGCATGATCGAACACA
seqA R4	GCTCTAGATGCCTCCGGTTTCAGTACAT
ArcA F1	CGGGATCCCCCACGACCAAGCTAATGAT
ArcA R2	*CCGTGAATG*ATAAGAATGTGCGGGGTCTG
ArcA F3	*CATTCTTAT*CATTCACGGCGAAGGTTATC
ArcA R4	GCTCTAGAGTCCTGTGAGCATCCCCTTA
CpxR F1	CGGGATCCGCCATAACAGCAGCGGTAAC
CpxR R2	*AAACCACGG*CAGCTCTCGGTCATCATCAA
CpxR F3	*CGAGAGCTG*CCGTGGTTTAAAACATTGCGT
CpxR R4	GCTCTAGATCTCTACGCGGCCATATTTT
KdpE F1	CGGGATCCATAATGGGCCGGGTATTCCT
KdpE R2	*GGCGTGGGC*GGATGGCCTGTTCATCTTCA
KdpE F3	*AGGCCATCC*GCCCACGCCATTTTATTATC
KdpE R4	GCTCTAGAGCAATCTGTGAACCAGATCAAC
PhoP F1	CGGGATCCTGACGCCGGCAAATTATATC
PhoP R2	*CGTGCGGAT*TCAGGTGGTGGCGTAATAA
PhoP F3	*ACCACCTGA*ATCCGCACGATGTCATTACC
PhoP R4	GCTCTAGAGCAGACGAAACGTGGTTTTA
SirA F1	CGGGATCCAAATAGCAGCCCGGAACAG

SirA R2	*CTCCGCATT*CACCAGTTCGTGGTCATCAA
SirA F3	*GAACTGGTG*AATGCGGAGACGTTAACAAGC
SirA R4	GCTCTAGAATGCGTTACCGTGACATCAA
BaeR F1	CGGGATCCACGGAAGTGTCCCGTAACC
BaeR R2	*CGTATAAAT*CTGTCCCAGCTTGGGTTCAT
BaeR F3	*CTGGGACAG*ATTTATACGCGCGGTCTACG
BaeR R4	GCTCTAGATAGCGGTGAGATGACGTTTC
DpiA F1	CGGGATCCAAGTACCCGAGCTGACGAAC
DpiA R2	*AATCAGGTG*GCGGATATATTCTGCGTGCAT
DpiA F3	*TATATCCGC*CACCTGATTGTCGCAGAGAT
DpiA R4	GCTCTAGACCGGGATGGCTAAAATATCA
CreB F1	CGGGATCCCAGGGAGAGGTGGTTTTCAA
CreB R2	*TAGTTTAGC*TCCCTGTTCATCCTCGACTA
CreB F3	*GAACAGGGA*GCTAAACTACGCGCGATCA
CreB R4	GCTCTAGAGGTTGCCGGAAAGGAGAT
HydG F1	CGGGATCCGATCAGCTGGAGGCGATT
HydG R2	*CACTGCACG*CGTGCAGTGGCTTACATCAT
HydG F3	*CAGCGTTTT*AAAACGCTGCTGGCTAAACT
HydG R4	GCTCTAGAACCAAACTCGCCGATGAC
NarL F1	CGGGATCCAAACGCCGAACGCAGTAAT
NarL R2	*CCAGACGCC*TTACACCCGTGCGTAGCAT

NarL F3	*CGGGTGTAA*GGCGTCTGGATATCACCGAAA
NarL R4	GCTCTAGACAGCGTGTCTCTTCCAATGA
NarP F1	CGGGATCCGCAGCTACATATGCCACACT
NarP R2	*AGCCCCTGC*ATACCTCGCCGCATAAGTGG
NarP F3	*GCGAGGTAT*GCAGGGGCTTTCTAACAAAC
NarP R4	GCTCTAGATGGCGCAGGAGAAATAAGAC
GlnG F1	CGGGATCCGCTGTTCTACCCGATGGTCA
GlnG R2	*ACCCTGCGT*CACCCAACGGATGGAACTAT
GlnG F3	*CGTTGGGTG*ACGCAGGGTCATAAACAGGA
GlnG R4	GCTCTAGACGCTCCACTCGATACCAGAT
YfhA F1 (*qseF*)	CGGGATCCTGGGGAGTTTAACCGACATT
YfhA R2 (*qseF*)	*GAGAGTAGC*TTCAGTAATCCGGGATCGTC
YfhA F3 (*qseF*)	*ATTACTGAA*GCTACTCTCCCGTCACGAA
YfhA R4 (*qseF*)	GCTCTAGACTTCAGTGACCGTCATACCG
RstA F1	CGGGATCCGGGCAGCTTTAATCACGAGT
RstA R2	*TCAGCAGCG*TAAGCGGCAATGAGAGAACC
RstA F3	*TGCCGCTTA*CGCTGCTGAGCCCTATCGTAT
RstA R4	GCTCTAGAAAGGGGCTGTTCCGACTAAT
assT F1	CGGGATCCTCGACTCACTCAGGCAATTA
assT R2	*TTATCCGAC*ACAACGACTGCGCCTAATTT
assT F3	*AGTCGTTGT*GTCGGATAAACCCAACCAGA

assT R4	GCTCTAGACGGCGACTTTATCAGAAACC
katG F1	CGGGATCCCTGCCGGGAGCTTTATTACA
katG R2	*GTTGGAACC*AAGATCCACACGAAGCTG
katG F3	*GTGGATCTT*GGTTCCAACTCCGTACTGC
katG R4	GCTCTAGAGTCGGCTTGCAATGAAAA
sodB F1	CGGGATCCCGCCACCTCTCAATTTGC
sodB R2	*GTTAACCAG*CACGTAAGTCTGATGATGTTTGC
sodB F3	*ACTTACGTG*CTGGTTAACTGGGAATTTGTTGC
sodB R4	GCTCTAGACCGACCTGAAATTTTCGTTG
cdtB F1	CGGGATCCGGCATTGCCAGAATAAAATCA
cdtB R2	*CTCGATCGA*CGGCCTCCTGTACCATAAGA
cdtB F3	*AGGAGGCCG*TCGATCGAGCACCTTATTCA
cdtB R4	GCTCTAGAGGCTCATAAACACGCCATT
ygaA F1	CGGGATCCTGCAGGTAGTGTGCCAACTT
ygaA R2	*CGTCGATTC*CACCTGACGCAGCGTAGTAA
ygaA F3	*CGTCAGGTG*GAATCGACGGAGAACTTCCA
ygaA R4	GCTCTAGAATGAAAACGGCAAACTCAC
cheY F1	CGGGATCCCGAAGCAAGTTGTGTGGTGT
cheY R2	*GTTTCTCAA*GCATGGTCGAAAAGTCATCC
cheY F3	*CGACCATGC*TTGAGAAACTGGGCATGTGA
cheY R4	GCTCTAGACCGTCATCTGGACGACATAA

ydiV F1	CGGGATCCCGTCTGTTTCCGTCCGTAGT
ydiV R2	*TTCGAATGA*AGGCGCTACCAACTCAGTC
ydiV F3	*GTAGCGCCT*TCATTCGAACCATTCATGC
ydiV R4	GCTCTAGACATAGACATAGGGCCTCCTGTCTTCA
BarA F1	CGGGATCCTTTCCGT TATGACGGGC TAC
BarA R2	*AGGTTCTTC*AGGCTGTAG TTGGTCAT GGA
BarA F3	*CTACAGCCT*GAAG AACCT TTGCC AGCTTA
BarA R4	AAGGAAAAAAGCGGCCGCGGGCAACAAT TACGCTGAGT
Bcf F1	CGGGATCCACTCACGACGTTGAGTAGCT
Bcf R2	*ACGTCATTC*TGACGGTTGTAGTATCCGCT
Bcf F3	*CAACCGTCA*GAATGACGTGGGAACCTTAG
Bcf R4	AAGGAAAAAAGCGGCCGCTGCTACGCGG TTCAGTCATA
CitB F1	CGGGATCCATTTTCGAGCGTGGAGTGAC
CitB R2	*TAGCTGATC*GCTAGCATCGGTTCGTCTTC
CitB F3	*GATGCTAGC*GATCAGCTACGGGAAAGTGG
CitB R4	GCTCTAGAACCCTGCAATCCTGTTTTGT
csg F	CGGGATCCTGGGGCTAATCTTTGGCTAT
csg R over	*AACTCAATC*TATGAAGTACAGGCAGGCGT
csg F over	*TACTTCATA*GATTGAGTTGTCTCGTCTTA
csg R	AAGGAAAAAAGCGGCCGCGCCTATGGCAGGGATATTTT
feoAB F1	TGCACATTAGAGGTATTTGCAA

feoAB R2	*CACGGCTGC*TGAATTGCATACGACCTACTT
feoAB F3	*TGCAATTCA*GCAGCCGTGTAGACATTGAA
feoAB R4	TTCAGCATGGCGTCGATCAT
FimAF	CGGGATCCGATATCGAAACCGGGTGTGT
FimAR-over	*ACCTACAGG*GCATATTGGTGCCTTC
Fim WF	GCTCTAGACTCAGCACGCATAAAGTG
FimWR-over	*ATACGCTGC*CCTGTAGGTATCGTTACT
flhC/D F1	CGGGATCCCTATGACAGGATGCGCAGTC
flhC/D R2	*CAGCGTTTG*TTGCGTGTAGTTTATGCCAG
flhC/D F3	*TACACGCAA*CAAACGCTGTGCAAGGAGTA
flhC/D R4	AAGGAAAAAAGCGGCCGCTGGGAAGCTGCGTTATACGT
LeuO F1	CGGGATCCTGAATCGCAATGGTGTGACT
LeuO R2	*TATCACGCC*GAAGCTGTGGTTTGCCCATA
LeuO F3	*CACAGCTTC*GGCGTGATAAAGGGCATCAA
LeuO R4	GCTCTAGAGTCTGAATCACACCAGGTAA
lrhA F	CGGGATCCTTGTATCTGTGCGTCTCGGT
lrhA R over	*GCACAACAT*TATCGGGCTGACCTGGTTAATGCT
lrhA F over	*AGCCCGATA*ATGTTGTGCCGCGATCCCAAT
lrhA R	GCTCTAGATTTCATCGCCAGCGGCTCTTT
ompR F	CGGGATCCGGGGTTGCCGATTAATTGTA
ompR over R	*TGTCGTCCC*ACGCATATCGTCATCAACCA

envZ over F	*GATATGCGT*GGGACGACAAAAGAGGCATA
envZ R	GCTCTAGATGGCGAAACTGTTCATTGAG
oxyR F1 rev	CGGGATCCTTATAAGCGTAGCGCCATCA
oxyR R2 rev	*CTCATAACG*CATAACGCCCAGCTCATCTT
oxyR F3 rev	*GGCGTTATG*CGTTATGAGCAACTGGCAGA
oxyR R4 rev	GCTCTAGAATCCCCACCGGGATTTATAC
pflA-STY0975 F1	GCTCTAGACAGCAGATAAGCGACTCTGT
pflA-STY0975 R2	*CGTCTGTCA*GACCATGGAACGTGTAAAGG
pflA-STY0975 F3	*TCCATGGTC*TGACAGACGCTCGAAGTATT
pflA-STY0975 R4	AAGGAAAAAAGCGGCCGCCGCGTTTCCGTACGACAA
RcsC F	GCTCTAGAGATCGTATGCGCCTGTTAGG
RcsC R over	*TACCGGGAA*GGCGATTAAGAGCCAAATGA
RcsD R over	*TTAATCGCC*TTCCCGGTAGAAGGGAGAAT
RcsD F	CGGGATCCGCGAAGGTTGTACGCTTTTC
invA BF	CGGGATCCCCTACAAGCATGAAATGGCAGAAC
invA BR	*AGGACAAGA*CTTCAATCAAGATAAGACGGCTGG
invA EF	*TGATTGAAG*TCTTGTCCTCCTTACGTCTGTCG
invA ER	GCTCTAGACGCCCAGATCCATACATCATCG
ssaU R	CGGGATCCAATACGCTATCTGGTGCTTG
ssaU F over	*TGTTTCGAC*TGCAGCCTTGTTACGTATGG
ssrB F over	*AAGGCTGCA*GTCGAAACACATCGGATGAAT

ssrB R	GCTCTAGAAGGCGTAAGGCTCATCAAAAT
StaAF	AACTGCAGGCGTGAAGAGGAAACTACTT
StaAR	GCTCTAGAAAATAATCATGGAACCAGTC
StaGF	AACTGCAGTTATCCAAAGCAGATTATGG
StaGR	CGGGATCCGCGTTAGCCTTTTCTGGGCA
TviA F	CGGGATCCCAATGAATTGTGCAGGTTTGA
TviA R-over	*CAATAACAG*CCTCATGAAGTCTCCTTATGCT
TviA F-over	*TTCATGAGG*CTGTTATTGCCGGATTCAGT
TviA R	GCTCTAGAAAGAATACGCTTTTTATTAACGTCG

*Restriction enzyme sites are underlined. Letter in italics represent overlapping sequences.

3.3 Expression de *std* entre *S.* Typhi et *S.* Typhimurium

Vu la forte expression de *std* chez *S.* Typhi, nous nous sommes intéressés à comparer son expression chez *S*. Typhimurium ou chez *E. coli* qui normalement ne code pas ce fimbria. Le vecteur d'expression utilisé précédemment (pRS415*std*) a été transformé vers SL1344 et DH5α.

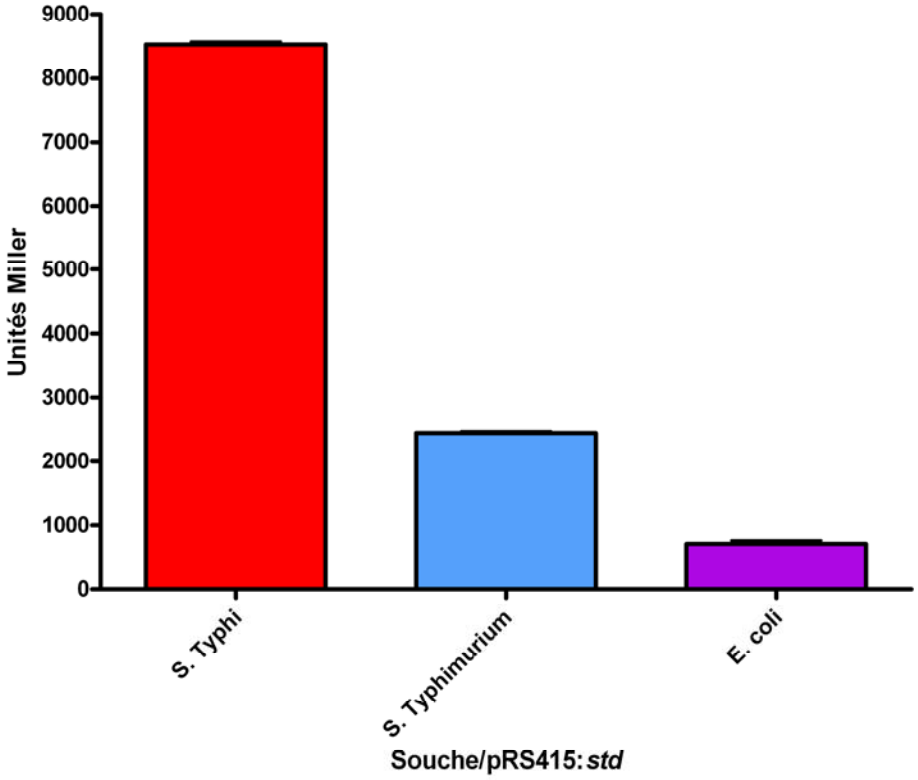

Figure 18. – Expression du promoteur *std* de *S*. Typhi chez différentes souches bactériennes.

Les résultats d'activité β-galactosidase sont présentés comme moyenne des unités Miller ± erreur type de la moyenne (± SEM) et sont représentatifs d'au moins 3 réplicats.

Une importante différence est notée entre les trois souches. Le promoteur *std* a la plus forte expression chez *S.* Typhi et la plus faible chez *E. coli*. Peu importe la provenance du promoteur *std* (*S*. Typhi ou *S*. Typhimurium), les résultats entre les promoteurs sont toujours similaires, c'est-à-dire que dès que la souche réceptrice du plasmide d'expression est *S*. Typhi, il y a une forte expression et vice versa chez *S*. Typhimurium (données non-montrées). Par la suite, nous avons vérifié que l'expression de *std* n'était pas dépendante des souches utilisées. Pour cette expérience, nous avons transformé le pRS415*std* dans différentes souches de *S.* Typhi (ISP1820, Ty2 et SARB63), de *S.* Typhimurium (SL1344 et 14028) et de *S.* Paratyphi A (SARB42).

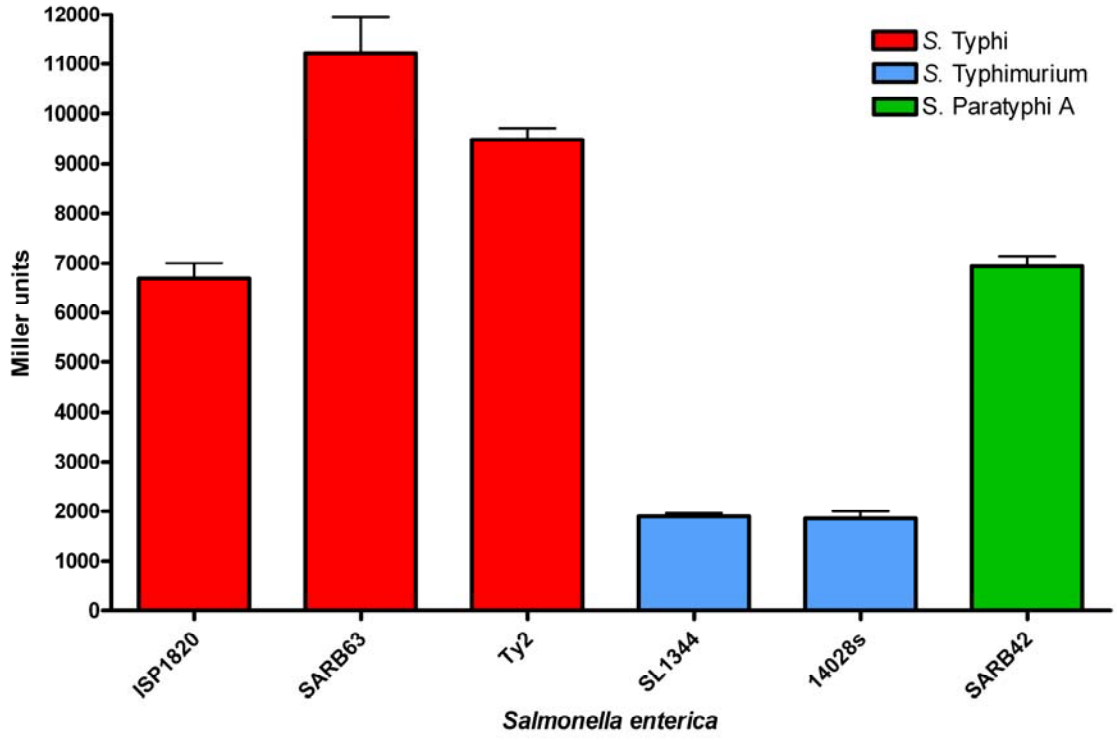

Figure 19. – Expression du promoteur *std* de *S*. Typhi chez différents sérovars de *S. enterica*.

Les résultats d'expression de *std* sont présentés comme moyenne des unités Miller ± erreur type de la moyenne (± SEM) et sont représentatifs d'au moins 3 réplicats.

Nous voyons que les souches de *S.* Typhi ont toujours la plus forte expression de *std* et que *S.* Paratyphi A suit de très près avec une expression du promoteur *std* très similaire à la souche ISP1820. Par contre, chez *S*. Typhimurium l'expression diminue de 3 à 4 fois comparativement à ISP1820. Il semble donc que l'expression de *std* est plus élevée chez *S.* Typhi comparativement à *S.* Typhimurium.

3.4 Criblage de régulateurs de l'expression de *std*

En plus de de cribler de possibles régulateurs, nous avons aussi cribler une banque de mutants par insertion de transposon Tn10 chez ISP1820 afin d'identifier des régulateurs uniques à *S*. Typhi qui expliquerait sa différence d'expression par rapport à *S*. Typhimurium. Le vecteur pRS415 avec la fusion promoteur *std-lacZ* (pRS415*std*) a été transformé dans la banque et les mutants ont été criblés sur LB avec Xgal. L'expression des mutants d'intérêt a été vérifiée par essai β-galactosidase contre la souche sauvage. La position de 17 transposons a été identifiée par utilisation d'une PCR nichée semi-aléatoire (''Nested semi-randomized PCR''), puis par séquençage Sanger.

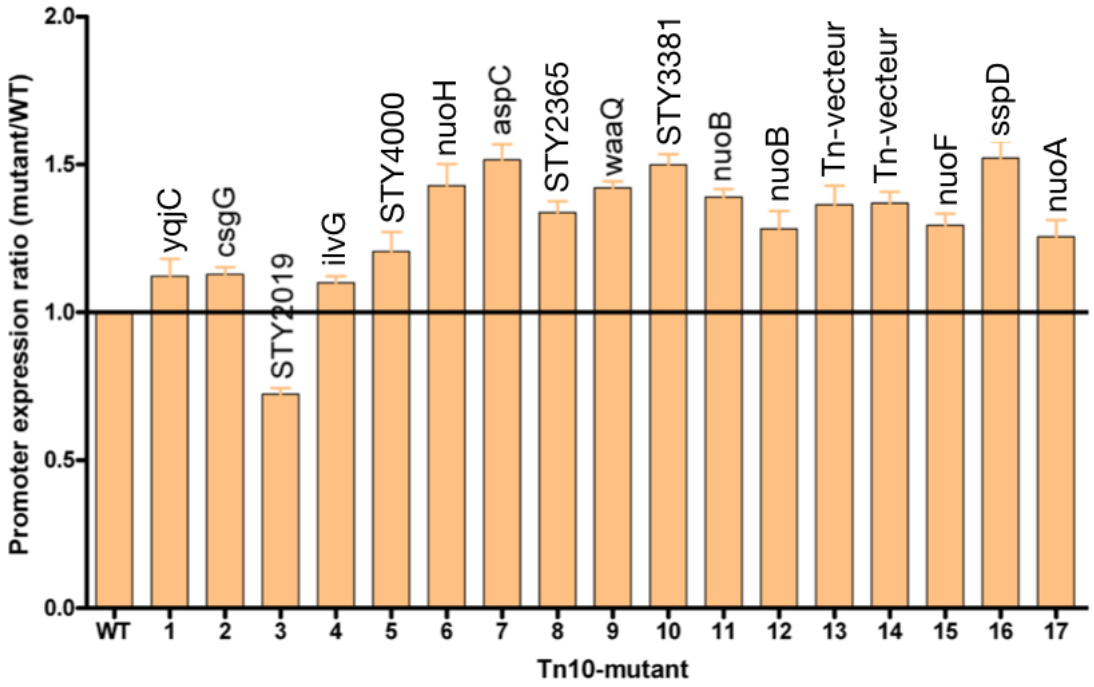

Figure 20. – Expression relative de *std* chez les mutants par insertion de transposon Tn10.

Les résultats sont présentés comme moyenne du ratio d'expression (unités Miller de la souche d'intérêt / unités Miller de la souche sauvage) ± SEM et sont représentatifs d'au moins 3 réplicats.

Parmi les gènes identifiés, seulement STY2019 avait une plus faible expression lorsque Tn10 y était inséré. Les 16 autres mutants avaient une plus forte expression. Par contre, les insertions 13 et 14 contiennent le vecteur complet et non simplement le transposon. 5 des transposons étaient insérés dans l'opéron *nuo* codant pour la NADH déshydrogénase de type 1.

Chapitre 4 – Régulation du fimbria Fim chez *Salmonella enterica* sérovar Typhi

4.1 Préface au chapitre

Fim est un fimbria de type 1, le type fimbriaire le plus étudié toutes espèces confondues. Chez *S.* Typhimurium, plusieurs gènes accessoires sont codés à la suite de l'opéron *fim* et le régulent. Plusieurs autres régulateurs ont été identifiés incluant Lrp, YaiV, YqiC et AdhE. Chez *S.* Typhi, nous avons précédemment identifié que le fimbria Fim avait un effet sur la formation de biofilm, l'interaction aux cellules hôtes, ainsi que sur la motilité. Vu cet effet majeur sur la virulence de *S.* Typhi, Fim est un candidat de choix pour l'étude de sa régulation. Nous avons donc voulu identifier les régulateurs de *fim* chez *S.* Typhi en testant différents régulateurs candidats et en criblant une banque de mutants par insertion de transposon. Plusieurs modulateurs de l'expression ont pu être révélés et un facteur a démontré une différence significative sur les phénotypes spécifiques au fimbria de type 1. Cet article sera soumis à un processus de révision par les pairs probablement dans ''Microbiology''.

Article 4 : Dufresne, K. et Daigle, F. 2020. Identification of novel regulators of Type 1 fimbriae (*fim*) in *Salmonella enterica* serovar Typhi

Contribution des auteurs :

J'ai réalisé l'élaboration du projet, les différentes expériences, l'écriture du manuscrit et la création des figures et tableaux. France Daigle m'a conseillé tout au long du processus et a fourni le support financier.

4.2 Article 4: Identification of novel regulators of Type 1 fimbriae (*fim*) in *Salmonella enterica* serovar Typhi

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Running title: Regulation of *fim* in *S.* Typhi

4.2.1 Abstract

Salmonella enterica serovar Typhi (*S.* Typhi) genome encodes 14 fimbrial gene clusters including a type 1 fimbria (T1F). This T1F is important for many steps of the pathogenesis of *S.* Typhi, however its regulation is poorly understood. In this work, we wanted to identify new regulators of *fim* expression. We used two different approaches for the identification of novel regulators: a candidate approach and a transposon-based screening. We identified 18 modulators of *fim* promoter expression including known T1F regulators in *S.* Typhimurium and novel identified regulators. We also confirmed that *ndh* mutant inhibits Fim-specific phenotypes (human cells adhesion and yeast agglutination). Overall, we observed major involvement of envelope integrity and electron transport chain on *fim* expression. Our results confirmed the importance of environmental cues for *S.* Typhi virulence.

4.2.2 Introduction

Salmonella enterica serovar Typhi genome encodes 14 adhesion systems called fimbriae. The chaperone-usher fimbriae of *Salmonella* present the most diverse fimbrial class and count 12 systems, including one type 1 fimbriae (T1F) named Fim [1].

T1F are the most extensively studied adhesion systems and the most common fimbriae in the Enterobacteriaceae family [2]. In *Escherichia coli* (*E. coli*), the *fim* operon is controlled by a region that switches from an OFF state to an ON state (phase variation). The expression is under the control of multiple regulators [3]. In *Salmonella*, Fim fimbriae are orthologous to Sfm fimbriae, another T1F of *E. coli*. *Salmonella* T1F are the only fimbriae expressed in laboratory conditions [4]. The *Salmonella fim* operon is followed and regulated by ancillary genes (*fimZ*, *fimY*, *fimW*, *STM0551* and *fimU*). FimZ is the major activator of the *fim* operon and when it is coupled to FimY, there is activation, but when it is associated with FimW, there is repression. Other than the ancillary genes, expression of the *fim* operon is modulated by a serie of factors including Lrp, YqiC, IprA (YaiV) and AdhE in *S*. Typhimurium [2, 5-8].

The *Salmonella* T1F are composed of repeated units of FimA, the major subunit, some minor subunits, FimF and by FimH, the adhesin. The FimH adhesin binds to high-mannose oligosaccharides on the eukaryotic extracellular glycoproteins [2]. Variants of FimH was observed in different serovars and resulted in difference in adhesion to host cells, in virulence in mice and in yeast agglutination [9, 10]. These FimH variants diverge only by few amino acids, but show huge difference in binding phenotype [10].

A fine equilibrium between expression of virulence factors allows *Salmonella* to effectively cause infection. This equilibrium is maintained by several regulators that modulate the expression of T1F and other virulence factors. Bacterial cells are exposed to numerous environmental stresses

in the intestinal tractus such as nutrient limitation, acidic environment, thermal stress, reactive oxygen species (ROS) and osmotic stress. For an enteric pathogen such as *Salmonella*, regulating its responses to environment is crucial for efficient virulence [2].

In this study, we aimed to identify regulators of *fim* expression in *S.* Typhi. We tested different candidate regulators and screened a library of mutants by insertion of transposon. We confirmed 18 mutants as factors modulating T1F expression. We also confirmed the role of Ndh on adherence to human cells and agglutination to yeast cells. Finally, we also discussed the importance of environmental signals for T1F expression in *S*. Typhi.

4.2.3 Materials and methods

4.2.3.1 Bacteria, plasmids and growth conditions

Strains and vectors are listed in Tableau 11. Bacteria were cultured on LB (Luria-Bertani) agar or LB broth. Experiments were generally performed in LB broth except if cited otherwise. Antibiotics or supplements were used at the following concentrations: 34 ug/ml chloramphenicol, 50 ug/ml kanamycin, 100 ug/ml ampicillin, 50 ug/ml diaminopimelic acid (DAP). Introduction of plasmid in specific strains was performed by electroporation [11].

4.2.3.2 Cloning of *fimA* promoter and chromosomal deletion of putative regulator genes

The fusion of the *fimA* promoter to the *lacZ* gene on pRS415 vector (pRS415:*fim*) was described in Dufresne, Saulnier-Bellemare [12]. Mutant strains of putative regulators were constructed by allelic exchange mutagenesis as described in Forest, Faucher [13]. Non-polar deletions were confirmed by PCR and Sanger sequencing (data not shown). Primers used in this study are listed in Tableau 12.

4.2.3.3 Screening of transposon-based library on MacConkey agar

The vector pRS415:*fim* was transformed in a transposon-based mutant library (Tn*10*-based library) constructed by Sabbagh et al [14]. The library was screened on MacConkey agar (Dibco) for the expression of the *fim* promoter. The wild-type strain harbouring the vector without (pRS415) or with *fim* promoter region (pRS415*fim*) were incubated simultaneously on MacConkey as negative and positive controls respectively. For each mutant isolated, the region in 3' of transposon was amplified by nested PCR using semi-random primers [15]. The primers used are designated *RB1-RNA*, *RB1-PCR*, *pLOF F seq* and *STY:PCRNiche#2* and are listed in Tableau 12. The PCR products were sequenced (IRIC) and identified with BLAST (NCBI).

4.2.3.4 β-galactosidase assays

Expression of the *fimA* promoter in the different strains was assessed by β-galactosidase assay as described in Miller [16]. Cells were grown in LB broth overnight at 37˚C with agitations. After cells lysis, β-galactosidase assay was performed using o-nitrophenyl-β-D-galactopyranoside (ONPG). Optical density at 420 nm and 550 nm were monitored and Miller units were calculated.

4.2.3.5 Adhesion assay

Henle cells (INT-407) were routinely maintained in EMEM with 10% FBS. Adhesion assay was adapted from Forest, Faucher [13]. The assay was performed with MOI of 1:20 after incubation overnight at 37˚C with agitation. The initial colony forming units (CFU) and CFU after 90 minutes of incubation (adhesion) were counted on LB agar plate. Adhesion rate was calculated as the ratio of adhesion CFU on initial CFU. Adhesion rate was presented with the WT strain at 100% of adhesion. The results represented at least three independent experiments.

4.2.3.6 Yeast agglutination

Concentrated bacteria were binary diluted for each strain tested and were incubated (1:1) with commercial yeast (Fleischman) in 24-well plate at room temperature. The presence of

agglutination was assessed for each strain by the greatest bacterial dilution with agglutination. The observations were performed directly and by stereoscope. The results are representative of at least three independent experiments.

4.2.3.7 Statistical Analysis

Statistical analysis was performed on GraphPad Prism. Two-tailed unpaired Student's *t*-test was applied on data sets for each construction compared to their control data. $P < 0.05$ was considered significant (*).

4.2.4 Results

4.2.4.1 Putative candidate regulators of *S.* Typhi *fim*

To identified putative *fim* regulators, we used *in silico* prediction (BPROM; Softberry) and tested for known regulators in *S.* Typhimurium, principally. Overall, 19 mutant strains were tested for *fim* expression (Figure 21A). Deletion of *fimZ*, *fimY*, f*imYZ*, *yqiC*, *crp*, *sirA* and *oxyR* causes lower expression and deletion of *fliZ*, *nagC*, *cpxR*, *ompR/envZ* and *lrhA* increase *fim* expression. FimW, Lrp, RpoN, GlnG, YaiV, ArcA and SoxRS cause no significant difference.

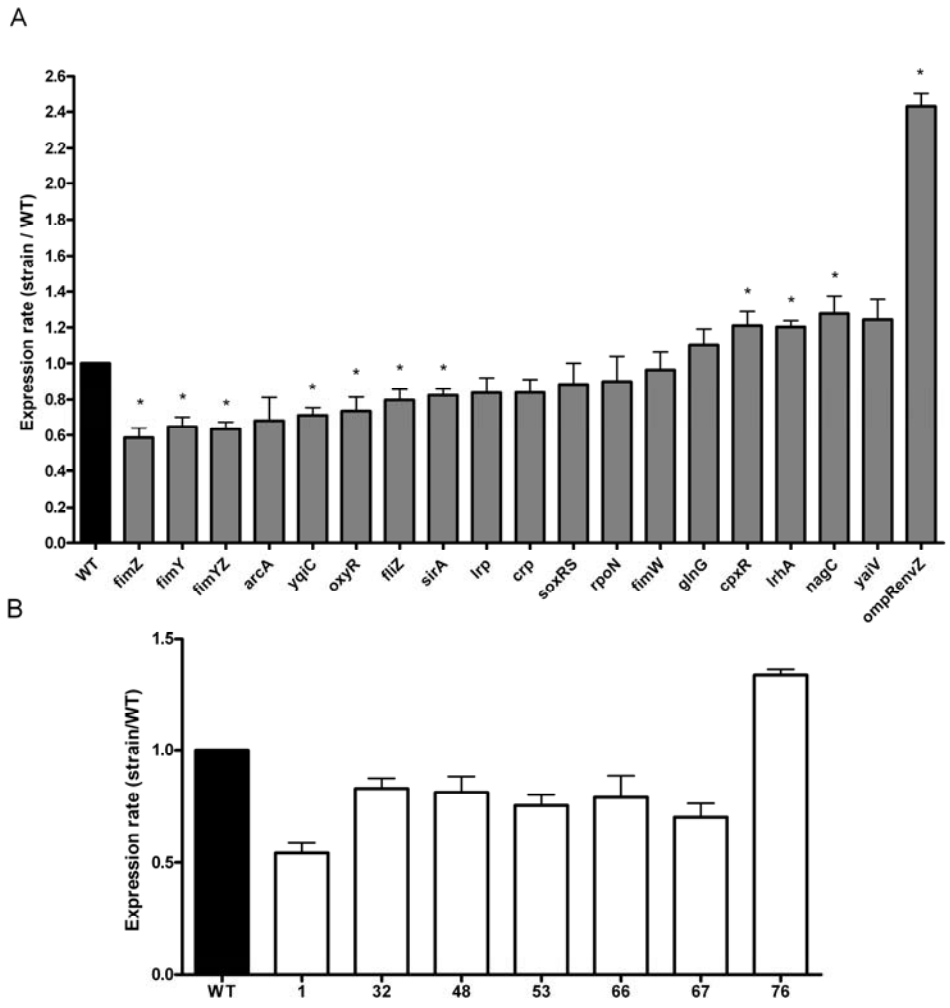

Figure 21. – Expression of *fim* promoter in putative regulators.

Expression of *fim* was evaluated by β-galactosidase assay. (A) Candidate regulators were compared to the WT strain. (B) Mutants from screening of the transposon-based library that have significant difference ($p<0,05$) compared to WT in β-galactosidase assay are presented. The results are showed as the mean of the expression rate ± SEM and are representative of at least 3 biological replicates.

4.2.4.2 Identification of novel *fim* regulators by transposon-based library screening

To discover novel regulators of *S.* Typhi fimbriae, the *fim* expression vector was transformed into a Tn*10*-based library of *S.* Typhi and the resulting colonies were screened on MacConkey agar. Seven candidates were confirmed to be different and were identified by sequencing (Figure 21B). The identification for the transposon positions is presented in Tableau 10. Among these strains, the *ndh* (FIM1) inactivation had the most significant phenotype on MacConkey and was similar to the negative control. The strains were also tested by β-galactosidase assay and seven of them have significant difference compared to positive control (Figure 21B). Here also, the *ndh* mutant has the most significant difference compared to WT strain.

Tableau 10. – Identification of transposon insertions for significant modulators of fim

Screening name	Gene with transposon insertion	Function
FIM1	*ndh*	Type 2 NADH dehydrogenase (NDH2)
FIM32	STY4579	Putative membrane protein
FIM48	*yeeF*	Putative transmembrane amino acid transporter
FIM53	STY1861 *(yddO)*	ATP-binding cassette domain-containing protein
FIM66	*tviE*	Vi polysaccharide biosynthesis protein
FIM67	*celD*	Putative *cel* operon repressor
FIM76	*waaK (rfaK)*	Lipopolysaccharide 1,2-N-acetylglucosaminetransferase

4.2.4.3 Ndh activates Fim specific phenotypes

Ndh is an inner membrane protein and it may indirectly activate *fim* by acting on its regulators. We have investigated the role of Ndh in two assays associated with T1F expression, yeast agglutination and adhesion to epithelial cells (Figure 22).

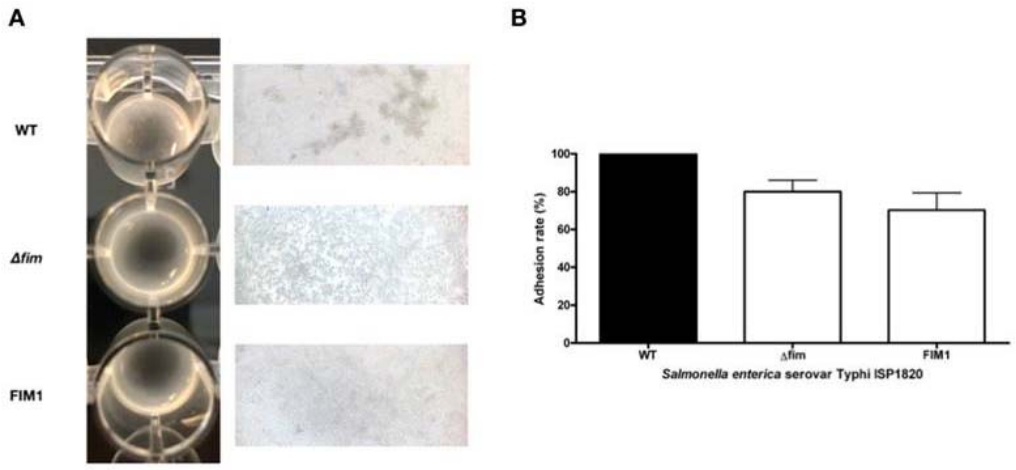

Figure 22. – Ndh affects Fim-specific phenotypes.

Two Fim-specific phenotypes, epithelial cells adhesion and yeast agglutination, were tested. (A). Yeast agglutination in 24-wells plate. Commercial yeast and bacterial strains are incubated together for 30 minutes and then observed. Visible phenotypes (left) and phenotypes observed by stereoscope (right) are showed. (B). Adhesion to INT407 is presented as the rate of adhesion where WT is 100% adhesion and other strains are compared to WT. Results are presented as the mean of adhesion rate ± SEM and are representative of at least 3 replicates.

Yeast agglutination of the *ndh* mutant was similar to the mutant harboring a complete deletion of the type 1 fimbria (*Δfim*) and has no agglutination or poor agglutination in the first dilution (1/2) with yeast (Figure 22A). The WT strain showed agglutination in every dilution tested. The other six mutants with significant difference in β-galactosidase activity did not demonstrate any difference compared to WT in the yeast agglutination experiment (data not shown).

Adhesion to epithelial cells showed that the *ndh* mutant (FIM1) was similar to the *fim* (*Δfim*) and had 80% adhesion level of the WT. The other strains of the screening were not tested as they did not present difference in yeast agglutination (Figure 22B).

4.2.5 Discussion

To identify novel factor influencing type 1 fimbria in *S.* Typhi, we tested promoter activity of *fim* in several mutant strains of regulator and we screened random mutant of a transposon-based library. Eighteen regulators of *fim* expression were identified. We confirmed that FimY and FimZ act as activators of *fim* operon in *S.* Typhi. However, FimW did not inhibit *fim* expression in *S*. Typhi compared to *S*. Typhimurium where it acts on *fim* operon by inactivation of FimZ. Also, YqiC acts as an activator in *S.* Typhi, which is opposite of *S.* Typhimurium SL1344. YqiC is involved in ubiquinone synthesis and acts as inhibitor of *fimZ* and *fim* operon [7]. Transcriptional factors predicted to bind to the promoter region of *fim*, were identified such as NagC that acts as an inhibitor and OxyR as an activator of *fim* expression. However, Lrp did not affect *fim* promoter in *S.* Typhi and may be involved in regulation of *fim* in other experimental conditions [6]. Regulators of flagella (FliZ, LrhA) and SPI-1 (SirA) modulate *fim* expression demonstrating link between fimbriae, flagella and invasion in *S.* Typhi. Response to nitrogen starvation did not act on T1F as two factors of nitrogen metabolism, RpoN and GlnG, did not show significant difference on *fim* expression.

Perturbations of bacterial envelope, sensed and regulated by many factors such as the two-component systems OmpR/EnvZ and CpxR, inhibit *fim* expression. Also, many extracellular components (TviE and WaaK) and transmembrane proteins (Ndh, STY4579 and YeeF) modulate *fim* expression. In case of envelope cell perturbation, the bacteria try to maintain its integrity and synthesis of fimbriae creates more instability: the bacteria then repressed its fimbrial operons. In brief, envelope stability is a key factor for *fim* activation in *S*. Typhi.

Several tested factors are involved in electron transport chain (ETC), aerobic respiration and regulation of oxidative stress (YqiC, OxyR, YaiV, SoxRS and ArcA). Also, FIM1 (Ndh) is a NADH dehydrogenase and one of the first electron acceptor of ETC and relay the electron to the quinones pool in aerobic conditions. Ndh is a major factor for aerobic respiration in bacteria and rules the balance between NADH and NAD+ [17, 18]. It produces reactive oxygen species (ROS) as results of its activity. Ndh must not be a direct regulator of *fim* promoter as it is inserted in inner membrane of the envelope and must affect different regulators sensing imbalance in ETC dependent of Ndh activity. Two possibilities are plausible: Ndh may affect ETC which bring respiration/metabolic impairs or it may cause membrane instability when absent that cause OmpR/EnvZ or CpxR activation. Altogether, Ndh and YqiC have similar activation effect on *fim* and are accountable for two adjacent components of ETC (NADH dehydrogenase and ubiquinone). Another interesting result is that OxyR, major regulator of oxidative stress by sensing peroxide in particularly, present similar *fim* expression when deleted than mutants for *ndh* and *yqiC*. Aerobic respiration activates ETC as oxygen can be the first electron donor and it is known that oxygen utilization brings reactive oxygen species during that process [19]. In summary, ETC is involved in *fim* expression by perturbation of the bacterial envelope or by its own specific activity.

In conclusion, we investigated the role of known regulators and screened a transposon-based library to identified genes involved in *fim* regulation. We established that envelope perturbations repressed *fim* expression in *S.* Typhi and that different levels of ETC are involved in *fim* activation. The role of ETC in *fim* expression should be further studied. Better understanding of *fim* regulation

will definitively allow a better comprehension of *S.* Typhi pathogenesis as Fim is one of the most important fimbria of *S.* Typhi and has an entire regulon involving several virulence factors.

4.2.6 References

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Tableau 11. – Bacterial strains and plasmids used in this study

Strain or plasmid	Name	Characteristic	Source or reference
		***S.* Typhi**	
DEF1045	WT	ISP1820 wild-type	R. Curtiss III, U. Florida
DEF154	*Δfim*	ISP1820 Δ*fimAICDHFZYXW*	(Dufresne et al, 2018)
DEF1153	pRS	WT (pRS415)	(Dufresne et al, 2018)
DEF1095	pRS*fim*	WT (pSIF474)	(Dufresne et al, 2018)
DEF1397	pRS Cm^r	WT (pRS415 Cm^r)	This study
DEF1398	pRSfim Cm^r	WT (pSIF519)	This study
TRASH44	FIM1	ISP1820 *ndh*::Tn*10* (pSIF519)	This study
TRASH54	FIM32	ISP1820 *STY4579*::Tn*10* (pSIF519)	This study
TRASH55	FIM48	ISP1820 *yeeF*::Tn*10* (pSIF519)	This study
TRASH58	FIM53	ISP1820 *yddO*::Tn*10* (pSIF519)	This study
TRASH68	FIM66	ISP1820 *tviE*::Tn*10* (pSIF519)	This study
TRASH60	FIM67	ISP1820 *celD*::Tn*10* (pSIF519)	This study
TRASH61	FIM76	ISP1820 *waaK*::Tn*10* (pSIF519)	This study
DEF1309	*ΔfimZ*	ISP1820 Δ*fimZ*	This study
DEF1316	*ΔfimY*	ISP1820 Δ*fimY*	This study
DEF1337	*ΔfimYZ*	ISP1820 Δ*fimYZ*	This study
DEF1239	*ΔarcA*	ISP1820 Δ*arcA*	(Murret-Labarthe et al, 2019)

DEF1328	*ΔyqiC*	ISP1820 Δ*yqiC*	This study
DEF1526	*ΔoxyR*	ISP1820 Δ*oxyR*	(Dufresne et al, 2020)
DEF1330	*ΔfliZ*	ISP1820 Δ*fliZ*	This study
DEF1247	*ΔsirA*	ISP1820 Δ*sirA*	(Murret-Labarthe et al, 2019)
DEF1329	*Δlrp*	ISP1820 Δ*lrp*	(Dufresne et al, 2020)
DEF1342	*Δcrp*	ISP1820 Δ*crp*	(Dufresne et al, 2020)
DEF884	*ΔsoxRS*	ISP1820 Δ*soxRS*	(Dufresne et al, 2020)
DEF1512	*ΔrpoN*	ISP1820 Δ*rpoN*	(Dufresne et al, 2020)
DEF1338	*ΔfimW*	ISP1820 Δ*fimW*	This study
DEF1307	*ΔglnG*	ISP1820 Δ*glnG*	(Murret-Labarthe et al, 2019)
DEF1238	*ΔcpxR*	ISP1820 Δ*cpxR*	(Murret-Labarthe et al, 2019)
DEF432	*ΔlrhA*	ISP1820 Δ*lrhA*	(Dufresne et al, 2020)
DEF1336	*ΔnagC*	ISP1820 Δ*nagC*	(Dufresne et al, 2020)
DEF1693	*ΔyaiV*	ISP1820 Δ*yaiV*	This study
DEF863	*ΔompRΔenvZ*	ISP1820 Δ*ompRΔenvZ*	(Murret-Labarthe et al, 2019)
DEF1345	*ΔfimZ*/pRS*fim*	*ΔfimZ* (pSIF474)	This study
DEF1346	*ΔfimY*/pRS*fim*	*ΔfimY* (pSIF474)	This study
DEF1347	*ΔfimYZ*/pRS*fim*	*ΔfimYZ* (pSIF474)	This study
DEF1697	*ΔarcA*/pRS*fim*	*ΔarcA* (pSIF474)	This study
DEF1350	*ΔyqiC*/pRS*fim*	*ΔyqiC* (pSIF474)	This study
DEF1530	*ΔoxyR*/pRS*fim*	*ΔoxyR* (pSIF474)	This study

DEF1349	*ΔfliZ*/pRS*fim*	*ΔfliZ* (pSIF474)	This study
DEF1446	*ΔsirA*/pRS*fim*	*ΔsirA* (pSIF474)	This study
DEF1351	*Δlrp*/pRS*fim*	*Δlrp* (pSIF474)	This study
DEF1352	*Δcrp*/pRS*fim*	*Δcrp* (pSIF474)	This study
DEF1724	*ΔsoxRS*/pRS*fim*	*ΔsoxRS* (pSIF474)	This study
DEF1608	*ΔrpoN*/pRS*fim*	*ΔrpoN* (pSIF474)	This study
DEF1348	*ΔfimW*/pRS*fim*	*ΔfimW* (pSIF474)	This study
DEF1607	*ΔglnG*/pRS*fim*	*ΔglnG* (pSIF474)	This study
DEF1445	*ΔcpxR*/pRS*fim*	*ΔcpxR* (pSIF474)	This study
DEF1677	*ΔlrhA*/pRS*fim*	*ΔlrhA* (pSIF474)	This study
DEF1353	*ΔnagC*/pRS*fim*	*ΔnagC* (pSIF474)	This study
DEF1698	*ΔyaiV*/pRS*fim*	*ΔyaiV* (pSIF474)	This study
DEF1473	*ΔompRΔenvZ*/ pRS*fim*	*ΔompRΔenvZ* (pSIF474)	This study
		***E.* coli**	
DEF1162	MGN-617	SM10 λpir asd thi thr leu tonA lacY supE recA RP4 2-Tc : :Mu[λpir] *asdA4*	(Kaniga *et al*., 1998)
pSIF064	pMEG*fim*	pMEG-375 with flanking region of *fim* operon used for fim operon deletion	(Dufresne et al, 2018)
		Plasmids	
pMEG-375		*sacRB mobRP4 oriR6K*, Cm^r Ap^r	R. Curtiss III, U. Florida
pSIF064	pMEG*fim*	pMEG-375 with flanking region of *fim* operon used for *fim* operon deletion	(Dufresne et al, 2018)
pSIF493	pMEG*fimZ*	pMEG-375 with flanking region of *fimZ* gene used for *fim*Z deletion	This study
pSIF494	pMEG*fimY*	pMEG-375 with flanking region of *fimY* gene used for *fimY* deletion	This study

pSIF499	pMEG*fimYZ*	pMEG-375 with flanking region of *fimYZ* gene used for *fimY*Z deletion	This study
pSIF475	pMEG*arcA*	pMEG-375 with flanking region of *arcA* gene used for *arcA* deletion	(Murret-Labarthe et al, 2019)
pSIF496	pMEG*yqiC*	pMEG-375 with flanking region of *yqiC* gene used for *yqiC* deletion	This study
pSIF535	pMEG*oxyR*	pMEG-375 with flanking region of *oxyR* gene used for *oxyR* deletion	(Dufresne et al, 2020)
pSIF495	pMEG*fliZ*	pMEG-375 with flanking region of *fliZ* gene used for *fliZ* deletion	This study
pSIF479	pMEG*sirA*	pMEG-375 with flanking region of *sirA* gene used for *sirA* deletion	(Murret-Labarthe et al, 2019)
pSIF503	pMEG*lrp*	pMEG-375 with flanking region of *lrp* gene used for *lrp* deletion	(Dufresne et al, 2020)
pSIF504	pMEG*crp*	pMEG-375 with flanking region of *crp* gene used for *crp* deletion	(Dufresne et al, 2020)
pSIF280	pMEG*soxRS*	pMEG-375 with flanking region of *soxRS* gene used for *soxRS* deletion	(Dufresne et al, 2020)
pSIF551	pMEG*rpoN*	pMEG-375 with flanking region of *rpoN* gene used for *rpoN* deletion	(Dufresne et al, 2020)
pSIF500	pMEG*fimW*	pMEG-375 with flanking region of *fimW* gene used for *fimW* deletion	This study
pSIF485	pMEG*glnG*	pMEG-375 with flanking region of *glnG* gene used for *glnG* deletion	(Murret-Labarthe et al, 2019)
pSIF476	pMEG*cpxR*	pMEG-375 with flanking region of *cpxR* gene used for *cpxR* deletion	(Murret-Labarthe et al, 2019)
pSIF107	pMEG*lrhA*	pMEG-375 with flanking region of *lrhA* gene used for *lrhA* deletion	(Dufresne et al, 2020)
pSIF501	pMEG*nagC*	pMEG-375 with flanking region of *nagC* gene used for *nagC* deletion	(Dufresne et al, 2020)
pSIF590	pMEG*yaiV*	pMEG-375 with flanking region of *yaiV* gene used for *yaiV* deletion	This study
pSIF283	pMEG *ompRenvZ*	pMEG-375 with flanking region of *ompR/envZ* genes used for *ompR/envZ* deletion	(Murret-Labarthe et al, 2019)
pRS415	pRS415	Multicopy vector with a promotorless, *lacZ* reporter gene, Ap^r	(Simons *et al.*, 1987)
pSIF518	pRS415 Cm^r	Multicopy vector with a promotorless, *lacZ* reporter gene, Cm^r Ap^s	This study

pSIF474	pRS415*fim*	pRS415 carrying the promoter region of fim*A*	(Dufresne et al, 2018)
pSIF519	pRS415*fim* Cm^r	pRS415 Cm^r carrying the promoter region of fim*A*	This study

Tableau 12. – Primers used in this study

Primers	**Sequence (5'-3')***
RB1-RNA	CGGGATCCAGCTTCTCACGCANNNNNNNNN
RB1-PCR	CGGGATCCAGCTTCTCACGCA
pLOF F seq	CAAGACGTTTCCCGTTGAAT
STY:PCRNiche#2	CTTGTGCAATGTAACATCAGAG
fimZ F1	CGGGATCCTCCACCGTGTGGTGTGTAG
fimZ R2	*CAAGTTTAG*CAACAGGATAAGTGCGCAGAT
fimZ F3	*ATCCTGTTG*CTAAACTTGGCCTTCACTC
fimZ R4	GCTCTAGATGCTACCCTGAAATTCTATGCG
fimY F1	CGGGATCCGATGGAAGGCATTGAAACATCA
fimY R2	*AGAAAGCTT*GAAATGATACCAACCGGCAAG
fimY F3	*TATCATTTC*AAGCTTTCTTCGGCTGATCC
fimY R4	GCTCTAGAAGATGTTGACGCTGGAGA
fimYZ R2	*CAAGTTTAG*GAAATGATACCAACCGGCAAG
fimYZ F3	*TATCATTTC*CTAAACTTGGCCTTCACTC
fimW F1	CGGGATCCTCTCAGCACGCATAAAGTGG
fimW R2	*TTTAACATG*GTCAATTTTCTGCTGCCAT
fimW F3	*AAAATTGAC*CATGTTAAAACGGAGCAGCAGT
fimW R4	GCTCTAGAGTGCCACCAAACACTCCTTC
fliZ F1	CGGGATCCTCAAACGATTACGCACCAA
fliZ R2	*CGCAGTGCG*GTAATGCGGTCGAGCAATTT
fliZ F3	*CCGCATTAC*CGCACTGCGTAAATACCA
fliZ R4	GCTCTAGACGTCCCAGCAGTGCTAATTT
yqiC F1	CGGGATCCCCGTGATGAGAAATGCGTTA
yqiC R2	*GCTTGTCGC*CGAACTCGCGAATACCTTT
yqiC F3	*GCGAGTTCG*GCGACAAGCCTGAAGAAGTAA
yqiC R4	GCTCTAGAGCAATATGGACGAGGAGCAC
YaiV F1	CGGGATCCCTGGTCTGCAAACGTTGGTG
YaiV R2	*TTCGTTGAT*CAAACTCGAAACGCGTACCA
YaiV F3	*TCGAGTTTG*ATCAACGAACGCGCGTATC
YaiV R4	GCTCTAGATGAGCTAAGCGGCAATAAACC

crp F1	CGGGATCCTGCAACCTCAGAGACAGTG
crp R2	*CCCATCCGG*CACGGAGCCTTTAACGATGT
crp F3	*GGCTCCGTG*CCGGATGGGATGCAGATCA
crp R4	GCTCTAGAGCCACATCGATGCAAACAAA
rpoN F1	CGGGATCCCCTGCAAGACGAACACGTTA
rpoN R2	*CTCAGTGGT*TCAAGCAGCGGGTTATTTTC
rpoN F3	*GCTGCTTGA*ACCACTGAGCGACAGCAAG
rpoN R4	GCTCTAGAGCAGGGCTTCAGTAATTTCG
lrp F1	CGGGATCCCCGGGCTAGTGAAATCTACG
lrp R2	*GGTGTCGTT*CCCATCCTTTTGCAGTTCAT
lrp F3	*AAGGATGGG*AACGACACCCGAACTTACG
lrp R4	GCTCTAGACTCTCCAGGTTCCAGGCTTT
nagC F1	CGGGATCCGGTGACGAGGTCGTTGACTT
nagC R2	*ACGGTGATC*AGAGGCCTGCTGATCGACT
nagC F3	*CAGGCCTCT*GATCACCGTTCTGCTATCG
nagC R4	GCTCTAGAGACGGGTAATTGGTCAGCA
soxRS F	CGGGATCCGCAGTATTGTCAGGGATGGC
soxRS over R	*AAGCAGCCG*ACTCCCAGCGATTACCGTCA
soxRS over F	*GCTGGGAGT*CGGCTGCTTGAAGATGATTA
soxRS R	GCTCTAGACAGTATCAACACAAACCGGA
ArcA F1	CGGGATCCCCCACGACCAAGCTAATGAT
ArcA R2	*CCGTGAATG*ATAAGAATGTGCGGGGTCTG
ArcA F3	*CATTCTTAT*CATTCACGGCGAAGGTTATC
ArcA R4	GCTCTAGAGTCCTGTGAGCATCCCCTTA
CpxR F1	CGGGATCCGCCATAACAGCAGCGGTAAC
CpxR R2	*AAACCACGG*CAGCTCTCGGTCATCATCAA
CpxR F3	*CGAGAGCTG*CCGTGGTTTAAAACATTGCGT
CpxR R4	GCTCTAGATCTCTACGCGGCCATATTTT
SirA F1	CGGGATCCAAATAGCAGCCCGGAACAG
SirA R2	*CTCCGCATT*CACCAGTTCGTGGTCATCAA
SirA F3	*GAACTGGTG*AATGCGGAGACGTTAACAAGC
SirA R4	GCTCTAGAATGCGTTACCGTGACATCAA

GlnG F1	CGGGATCCGCTGTTCTACCCGATGGTCA
GlnG R2	*ACCCTGCGT*CACCCAACGGATGGAACTAT
GlnG F3	*CGTTGGGTG*ACGCAGGGTCATAAACAGGA
GlnG R4	GCTCTAGACGCTCCACTCGATACCAGAT
FimAF	CGGGATCCGATATCGAAACCGGGTGTGT
FimAR-over	*ACCTACAGG*GCATATTGGTGCCTTC
Fim WF	GCTCTAGACTCAGCACGCATAAAGTG
FimWR-over	*ATACGCTGC*CCTGTAGGTATCGTTACT
lrhA F	CGGGATCCTTGTATCTGTGCGTCTCGGT
lrhA R over	*GCACAACAT*TATCGGGCTGACCTGGTTAATGCT
lrhA F over	*AGCCCGATA*ATGTTGTGCCGCGATCCCAAT
lrhA R	GCTCTAGATTTCATCGCCAGCGGCTCTTT
ompR F	CGGGATCCGGGGTTGCCGATTAATTGTA
ompR over R	*TGTCGTCCC*ACGCATATCGTCATCAACCA
envZ over F	*GATATGCGT*GGGACGACAAAAGAGGCATA
envZ R	GCTCTAGATGGCGAAACTGTTCATTGAG
oxyR F1 rev	CGGGATCCTTATAAGCGTAGCGCCATCA
oxyR R2 rev	*CTCATAACG*CATAACGCCCAGCTCATCTT
oxyR F3 rev	*GGCGTTATG*CGTTATGAGCAACTGGCAGA
oxyR R4 rev	GCTCTAGAATCCCCACCGGGATTTATAC

*Restriction enzyme sites are underlined. Letter in italics represent overlapping sequences.

Chapitre 5 – Discussion

S. Typhi est encore à ce jour une bactérie peu connue et la plupart des caractéristiques et informations qui lui sont attribuées sont habituellement extrapolées à partir du modèle murin d'infection systémique par *S.* Typhimurium. Par contre, la communauté scientifique s'intéresse de plus en plus à cette bactérie humain-spécifique et plusieurs données d'intérêt ont émergé au cours des dernières années. Il est maintenant évident que *S.* Typhi et *S.* Typhimurium, malgré leurs grandes homologies génétiques, sont très différentes dans leur régulation et leur virulence.

5.1 Caractérisation des fimbriae de type chaperon-placier chez *S.* Typhi

Notre laboratoire s'intéresse particulièrement aux systèmes d'adhésion de *S.* Typhi qui possède 14 fimbriae putatifs dont 12 de type chaperon-placier. Ces 12 fimbriae occupent une fonction commune, c'est-à-dire l'adhésion, mais jouent un rôle distinct au cours de l'infection et possèdent une spécificité d'interaction aux cellules hôtes ou autres surfaces d'attachement. Certains sont communs avec *S.* Typhimurium (Bcf, Fim, Saf, Stb, Stc, Std, Sth et Csg), tandis que d'autres sont uniques à *S.* Typhi (Sef, Sta, Ste, Stg, Tcf et Pil). De plus, 5 fimbriae possèdent un ou des pseudogènes dans leur opéron (Bcf, Sef, Ste, Stg et Sth) empêchant possiblement la formation d'un fimbriae fonctionnel.

Au chapitre 2, nous avons voulu caractériser fonctionnellement les fimbriae de type chaperon-placier. Nous avons voulu évaluer l'expression des 12 fimbriae de type chaperon-placier en conditions de culture mimant différentes étapes de l'infection par *S.* Typhi. Nous voulions identifier quel système produisait des fimbriae à la surface de la bactérie, et déterminer le rôle de chacun dans différentes étapes connues de la pathogenèse de *S.* Typhi.

Premièrement, notre caractérisation a permis de déterminer que les promoteurs fimbriaires étaient toujours plus exprimés en milieu minimal qu'en tout autres conditions rencontrées

(induction de SPI-1, induction de SPI-2, milieu riche ou milieu minimal). Il est très probable que les nutriments limités créent un stress chez la bactérie qui active les fimbriae de *S.* Typhi. Le profil d'expression des fimbriae était variable entre chacun, ce qui va de pair avec le fait que chaque fimbria a un rôle spécifique à jouer dans la virulence de *S.* Typhi et que chacun a son moment d'action et d'expression.

Deuxièmement, nous avons construit une souche afimbriaire pour les 14 systèmes d'adhésion de *S.* Typhi. Cette souche est devenue un outil important et puissant du laboratoire pour l'étude de tous les fimbriae. Puisque tous les fimbriae sont faiblement exprimés en laboratoire, nous avons induit chacun des systèmes fimbriaires dans la souche afimbriaire de *S.* Typhi de façon à déterminer la présence, mais aussi la structure de chacun des fimbriae. Nous avons pu observer pour la première fois chez *S.* Typhi 6 fimbriae (Fim, Saf, Sta, Stb, Std et Tcf), mais n'avons pu voir aucun des fimbriae possédant des pseudogènes dans leur opéron, ni Stc. Pour ce qui est des fimbriae possédant des pseudogènes, il est probable qu'il n'y ait peu ou pas de formation de fimbriae fonctionnel à la surface de la bactérie. Pour Stc, l'opéron est intact (sans pseudogène) et nous avons pu noter la présence d'une bande dans l'extrait de protéines extracellulaire présenté sur gel protéique. Cette bande concorde avec StcA, la sous-unité majeure du fimbria, et a été confirmée par spectrométrie de masse. Stc est donc présent à la surface de la bactérie, mais il est possible que le type de préparation que nous avons utilisé pour la microscopie électronique ne soit pas adaptée à la structure fimbriaire.

Finalement, cette étude s'est conclue en déterminant l'effet de chacun des fimbriae (délétion ou ajout d'un fimbria) dans la pathogenèse, y compris la motilité, la formation de biofilm et l'interaction avec 2 types cellulaires (cellules épithéliales et macrophages humains). Malgré que les fimbriae ne puissent pas tous être visibles à la surface de la bactérie, chacun présentait des variations lors des étapes de la pathogenèse qui ont été testées. Particulièrement, Fim présentait des différences phénotypiques à chacune des étapes testées, ce qui en fait un fimbria très important pour la virulence de *S.* Typhi. De façon intéressante, les fimbriae portant des

pseudogènes semblaient tout de même jouer un rôle dans la pathogenèse de *S.* Typhi sans être présents à la surface de la bactérie. Ces fimbriae possédant des pseudogènes pourraient jouer un rôle régulateur sur d'autres fimbriae au niveau transcriptionnel : il est possible que certains gènes de fonction inconnue à l'intérieur d'un opéron fimbriaire codent pour un modulateur non-identifié. Il est aussi possible que la partie fonctionnelle de l'opéron permettent la production de protéines pouvant ensuite jouer un rôle sur la pathogenèse de *S.* Typhi. Peu importe, *S.* Typhi a dans son génome 14 fimbriae encodés qui agissent directement ou indirectement sur les étapes majeures de sa pathogenèse.

En résumé, chaque fimbria a son importance sur la pathogenèse de *S*. Typhi soit en étant impliqué directement dans celle-ci, soit possiblement en modulant un autre fimbria. Nous avons donc une combinaison de 12 fimbriae qui mène à une infection efficace et tout reste à découvrir quant à leur expression et leurs cibles d'adhésion. De plus, l'impact des pseudogènes sur les opérons fimbriaires est un autre sujet d'intérêt à explorer davantage.

5.2 Régulation du fimbria Std

Comme Std était fortement exprimé, nous nous sommes intéressés à sa régulation. Chez *S*. Typhimurium, il a été démontré que l'opéron fimbriaire *std* est particulier du fait qu'en plus de coder toutes les composantes permettant la formation d'un fimbria fonctionnel, il porte en plus deux gènes codant pour des régulateurs. Ces deux régulateurs (StdE et StdF) sont reconnus chez *S.* Typhimurium pour gérer un régulon qui inclut plusieurs gènes de virulence. Nous croyons que certains des régulateurs connus chez *S.* Typhimurium sont aussi des facteurs affectant *std* chez *S.* Typhi et que plusieurs régulateurs restent encore à identifier. De plus, nous avons démontré qu'il y a une grande différence d'expression de *std* entre *S.* Typhi et *S.* Typhimurium (Section 3.3) et nous croyons que certains régulateurs spécifiques à *S.* Typhi expliquent la grande différence d'expression entre les deux sérovars. Premièrement, nous avons identifié une variété de régulateurs probables de *std*, soit par comparaison des régulateurs connus chez *S.* Typhimurium,

par prédiction bioinformatique ou par identification de régulateurs de virulence possibles. En tout, 53 mutants ont été construits et testés. Parmis ceux-ci, 21 facteurs démontraient une différence significative d'expression de *std* lorsque délétés. Les régulateurs connus chez *S.* Typhimurium avaient majoritairement un effet similaire chez *S.* Typhi à l'exception de SeqA et StdE. SeqA lie normalement des sites GATC hémi-méthylés. Le facteur Dam méthyle l'ADN aux sites GATC dès sa réplication et chaque souche bactérienne porte sa propre signature de méthylation. Il est possible que SeqA n'ait pas d'effet sur l'expression de *std* chez *S.* Typhi parce que le profil de méthylation des trois sites GATC du promoteur *std* est différent de celui de *S*. Typhimurium. Il est aussi possible que cette différence au niveau des sites GATC explique nos différences d'expression entre nos souches de *S.* Typhi (ISP1820, Ty2 et SARB63) et celles de *S.* Typhimurium (SL1344 et 14028) qui ont été testées au laboratoire (Section 3.3). De plus, il a été récemment démontré chez *S.* Typhimurium que HdfR activerait *std* en liant une section près des deuxième et troisième sites GATC du promoteur : si un de ces sites est méthylé, l'activation par HdfR est perdue. Si le patron de méthylation est différent de *S.* Typhimurium et que les sites GATC sont disponibles à l'attachement de HdfR chez *S.* Typhi, l'expression de *std* peut être plus élevée chez ce sérovar. En bref, un patron de méthylation différent entre *S.* Typhi et *S.* Typhimurium est une des possibilités envisagées quant à la différence d'expression de *std* entre *S*. Typhi et *S.* Typhimurium. En plus des régulateurs connus chez *S.* Typhimurium, nous avons voulu cribler une variété de régulateurs candidats, tant en identifiant des sites d'attachement au niveau du promoteur qu'en choisissant une variété de régulateurs de virulence, de métabolisme ou de réponse au stress. Parmi les régulateurs testés, plusieurs régulateurs du stress membranaire ont un effet inhibiteur sur l'expression de *std*. La formation d'un fimbria à la surface de la bactérie déstabilise elle-même l'enveloppe bactérienne en insérant son placier dans la membrane externe et en modifiant la perméabilité. En cas de stress membranaire, cette déstabilisation supplémentaire peut être fatale à la bactérie. De plus, plusieurs régulateurs choisis avaient un lien avec d'autres composantes de la virulence telles que la régulation des flagelles (FlhCD), de la capsule (TviA, RcsBCD), des systèmes de sécrétion de SPI-1 (HilD, BarA/SirA, RcsBCD) et -2 (BarA/SirA et PhoP). La plupart de ces facteurs de virulence sont eux-mêmes exprimés à la surface bactérienne, donc pourraient aussi déstabiliser l'enveloppe bactérienne. Par contre, une autre

possibilité est aussi mise de l'avant, c'est-à-dire que chaque facteur de virulence a son importance à un moment précis durant la pathogenèse et ceux-ci doivent être régulés différemment l'un de l'autre afin de permettre une infection optimale. Par exemple, les flagelles permettent le mouvement de la bactérie jusqu'à sa niche dans l'intestin humain, puis un fimbria permet l'adhésion à la surface intestinale et finalement le T3SS codé par SPI-1 doit agir pour l'invasion des cellules hôtes. Il est donc probable que certains facteurs de virulence interfèrent ou accentuent l'expression de *std*. La régulation de *std* et SPI-1 est intimement lié chez *S.* Typhimurium : les deux régulateurs codés sur l'opéron *std* (StdEF) ont eux-mêmes un effet sur l'expression de SPI-1 et les deux systèmes (Std et SPI-1) sont inversement régulés. Chez *S.* Typhi, le lien *std*/SPI-1 reste à investiguer et n'est pas direct. Quelques opérons fimbriaires (*fim*, *csg*, *sta* et *bcf*) ont aussi été testés et, de façon intéressante, Bcf semble moduler positivement *std*. Le gène codant pour le placier dans l'opéron *bcf* est pseudogène créant 2 codons d'arrêt prématurés dans le gène (175). Ceci renforce la possibilité que certains fimbriae possédant des pseudogènes aient un rôle pour soutenir la production des fimbriae fonctionnels et devrait être étudié davantage.

L'environnement bactérien est changeant et l'expression de *std* pourrait être liée à un changement métabolique, donc nous avons testé une variété de régulateurs métaboliques. Au final, il semble y avoir un lien entre la gestion de l'azote (ammonium) chez *S.* Typhi et l'expression de *std*. Un autre régulateur métabolique d'importance, c'est-à-dire Crp, serait quant à lui un activateur de *std*. Crp est responsable de la répression catabolique chez la bactérie et le récepteur de l'adénosine monophosphate cyclique (cAMP). La délétion pour *crp* présentait l'une des plus fortes diminutions d'expression du promoteur fimbriaire comparativement à la souche sauvage parmi les candidats régulateurs testés. Nous nous sommes donc intéressés à l'action de Crp sur *std* plus spécifiquement et avons pu déterminer que Crp agissait bien comme un activateur de *std* et ce en liant directement une région distale du promoteur qui possède le motif consensus (TGTGA-N6-TCACA). Crp semble aussi jouer le rôle d'activateur chez *S.* Typhimurium, ce qui en fait un régulateur commun aux 2 sérovars. En plus des expériences présentées au chapitre 3, il est considéré de démontrer *in vitro* l'attachement entre Crp et la région promotrice par un essai

de retard sur gel (''Electrophoretic mobility shift assay'' ou ''EMSA'') pour démontrer hors de tout doute l'attachement de ce facteur d'intérêt au promoteur de *std*.

Crp est le principal régulateur permettant l'utilisation de sucres différents du glucose comme source de carbone en activant les voies métaboliques qui permettent leur catabolisme (162). Crp a déjà été associé chez *S.* Typhi à la régulation de certains facteurs de virulence comme l'hémolysine (HlyE) par exemple (176). Crp a aussi été associé chez d'autres espèces à la régulation de fimbriae, soit au fimbria de type 1 chez *Serratia marcescens* et *Klebsiella pneumoniae*, soit aux fimbriae de type P chez *E. coli* uropathogénique (177, 178). Donc, en plus de capter les signaux reliés aux changements de source de carbone et d'activer/réprimer des gènes liés au métabolisme, Crp utilise ces signaux pour réguler la virulence. Vu les changements environnementaux que *S.* Typhi rencontre tout au long de sa pathogenèse à l'intérieur de l'humain, *std* sera donc exprimé en accord avec les sucres présents dans chacune des niches qu'offre le corps humain. Par exemple, le glucose, qui inactive Crp, est rapidement dégradé dès le début tractus gastro-intestinal. D'autres sucres d'intérêt sont présents dans le tractus intestinal et certains dérivent du métabolisme du microbiote, mais pourraient être utilisé par *S.* Typhi pour ses propres besoins. Il reste à investiguer la source de carbone qui permettrait une activation maximale de Crp et l'effet que cette activation aurait sur l'expression de *std*. Ceci permettrait de mieux comprendre quand Std est nécessaire à *S.* Typhi pour sa pathogenèse. Chez *S*. Typhimurium, Std lie des résidus fucosylés à la surface des cellules intestinales. Il est possible que Std joue un rôle similaire chez *S.* Typhi par reconnaissance de ces résidus comme sources de carbone par Crp. Std serait alors important au niveau intestinal pour l'adhésion initiale. Comme Std est présent chez la majorité des sérovars de *S. enterica*, il est très probable que le fimbria joue un rôle similaire entre *S.* Typhi et *S.* Typhimurium. Par contre, chez *S*. Typhi certains facteurs de virulence permettraient l'évasion du système immunitaire et le passage vers le sang, tandis que chez *S*. Typhimurium il y aurait persistance au niveau du colon principalement.

En plus de l'approche par régulateurs candidats, nous avons criblé une banque de mutants par insertion de transposon afin d'identifier de possibles nouveaux régulateurs de *std* (Section 3.4). Ces régulateurs pourraient être la clé de la différence d'expression de *std* entre *S.* Typhi et *S.* Typhimurium. En tout, 17 régulateurs présentaient une différence visible de coloration comparativement à la souche sauvage. Le seul gène semblant agir à titre d'activateur est un gène phagique (STY2019) codé sur le prophage ST18. Ce phage est présent chez *S.* Typhi, mais non chez *S.* Typhimurium, ce qui en fait un bon candidat comme activateur supplémentaire expliquant les différences d'expression entre les deux sérovars : l'impact de STY2019 sur l'expression globale de *std* reste à étudier. Plusieurs autres gènes ont été identifiés comme inhibiteurs et par 5 fois nous retrouvons des gènes de l'opéron *nuo* codant pour la NADH déshydrogénase de type 1 : *nuoA*, 2x *nuoB*, *nuoF* et *nuoH*. Nous croyons que la modulation de *std* par le complexe Nuo doit se faire de façon indirecte vu qu'il est inséré dans la membrane interne de la bactérie : ce sujet reste à investiguer davantage.

5.3 Régulation du fimbria Fim

S. Typhi code pour un fimbria de type 1 nommé Fim. Fim est conservé à travers tous les sérovars de *S. enterica*, mais absent chez *S. bongori*. Fim est reconnu pour lier des glycanes manosylés à la surface des cellules hôtes. Chez *S*. Typhi, il est impliqué à plusieurs niveaux dont la formation de biofilms, l'interaction aux cellules hôtes et la motilité bactérienne (Article 2). Vu l'importance de Fim comme facteur de virulence chez *S.* Typhi, nous nous sommes intéressés à sa régulation. Deux approches ont été utilisées pour déterminer les régulateurs de l'opéron *fim*, c'est-à-dire une approche par régulateurs candidats et une approche par criblage d'une banque de transposon.

Parmi les régulateurs criblés, 18 présentaient une différence significative. Certains modulateurs ayant un impact significatif sur *fim* étaient liés au métabolisme des sucres (Crp et NagC), au stress à l'enveloppe (SirA, CpxR et OmpR/EnvZ) ou au stress oxydatif (OxyR). Tout comme pour *std*, la perturbation de l'enveloppe semble avoir un effet inhibiteur sur l'expression de *fim*, possiblement relié au fait qu'une membrane instable ne peut se permettre l'insertion d'éléments supplémentaires pouvant la perturber. L'effet des régulateurs de l'enveloppe est possiblement

similaire pour la majorité des autres fimbriae de *S.* Typhi. Pour *fim*, plusieurs éléments transmembranaires ou extracellulaires ont aussi été retrouvés comme modulateurs (Article 4), démontrant de nouveau l'importance de l'enveloppe et son intégrité pour une expression optimale du fimbria. Par contre, seul *ndh* présentait un effet sur les phénotypes spécifiques au fimbria Fim tels que l'agglutination aux levures et l'adhésion aux cellules épithéliales. Cependant, Ndh est un élément inséré dans la membrane interne de l'enveloppe et ne peut pas agir directement sur la section promotrice de *fim*. Ndh code pour la NADH déshydrogénase de type 2 de *S.* Typhi et est impliquée dans les premières étapes de la chaîne de transport d'électron, ainsi que dans la balance NADH/NAD+ en condition aérobie. Deux possibilités sont envisagées pour expliquer l'effet activateur de Ndh sur *fim* : soit *fim* est régulé par des éléments de la chaine de transport d'électron et de la respiration, soit le retrait de Ndh provoque une déstabilisation de la membrane. Des résultats précédents, nous savons que OxyR, régulateur du stress oxydatif sentant le peroxide, a un effet sur *fim* et qu'un gène impliqué dans la synthèse des ubiquinones, c'est-à-dire YqiC, a aussi un effet d'activation. Par contre, SoxRS, régulateur du stress oxydatif sentant les radicaux hydroxyles, n'a pas d'effet significatif, ni ArcA, régulateur de la respiration aérobie. Notre hypothèse quant à l'effet de Ndh sur *fim* est que la protéine normalement présente provoque, lors de la respiration aérobie, une augmentation des espèces réactives à l'oxygène (principalement le peroxide) par une faible concentration en NADH et ainsi une activation de OxyR. OxyR lie alors directement le promoteur *fim* à un site d'attachement pour OxyR qui est retrouvé dans la section promotrice. Cette hypothèse reste à valider quant à l'effet du peroxide sur l'expression de *fim*, ainsi que la concentration de NADH/NAD+ chez le mutant *ndh*.

En conclusion, le fimbria Fim de *S.* Typhi est important à toutes les étapes de la pathogenèse précédemment testées. Il est inhibé par la perturbation de la membrane et son activation nécessite OxyR et la présence de stress oxydatif chez la bactérie.

Chapitre 6 – Conclusion

Les fimbriae de type chaperon-placier chez *S.* Typhi sont impliqués dans la pathogenèse de S. Typhi selon les conditions environnementales retrouvées. Nous avons pu caractériser chacun de ces fimbriae quant à leur expression en différentes conditions de culture, à leur présence à la surface de la bactérie et leur morphologie et quant à leur effet sur la pathogenèse de *S.* Typhi. Deux fimbriae se sont particulièrement démarqués, soit Std pour sa forte expression au niveau du promoteur et Fim quant à ces effets variés sur la pathogenèse de *S.* Typhi. Leur étude a été poursuivie pour identifier les facteurs régulant leur expression. Un nouveau facteur a pu être identifié et confirmé pour la régulation de *std*, c'est-à-dire Crp. Plusieurs facteurs d'intérêt sont identifiés dont des régulateurs d'autres facteurs de virulence, de la perturbation de l'enveloppe bactérienne et aussi quelques régulateurs métaboliques, dont principalement 2 régulateurs modulant l'acquisition d'azote (ammonium) par la bactérie, ainsi que le complexe de la NADH déshydrogénase de type 1. Pour *fim*, la perturbation de l'enveloppe semble aussi inhiber son expression et il reste à confirmer l'effet de la chaine de transport d'électron sur ce fimbria. Il est à noter que *fim* et *std* sont tous deux modulés par leur propre NADH déshydrogénase (*ndh* et *nuo*), ce qui ouvre la porte sur l'importance de la chaîne de transport d'électron sur la pathogenèse de *S.* Typhi par les fimbriae.

Il reste encore plusieurs mécanismes de régulation à étudier quant aux 12 fimbriae de type chaperon-placier chez *S.* Typhi. Une meilleure connaissance des signaux environnementaux activant ou inhibant leur action spécifique permettrait d'identifier s'il y a réellement redondance des systèmes ou si chaque fimbria joue un rôle à un moment précis de l'infection. Plus nous connaîtrons de détails sur leur régulation et mieux nous comprendrons la cascade d'événements qui se produit pour obtenir une infection efficace. Une autre voie pour la suite sur les fimbriae de *S.* Typhi serait l'étude des mécanismes entourant les fimbriae possédant des pseudogènes afin de comprendre leur importance malgré leur inactivation. En comprenant mieux les fimbriae de *S.*

Typhi, nous pourrions développer de meilleures thérapies contre ce microorganisme en contrant la virulence de la bactérie plutôt qu'en inhibant celle-ci (anti-virulent versus antibiotique). Les fimbriae sont aussi une bonne voie d'étude pour mieux comprendre la différenciation entre bactérie généraliste et bactérie hôte-spécifique vu leur combinaison qui varie d'un sérovar de *Salmonella* à l'autre et leur régulation qui varie aussi.

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Annexes

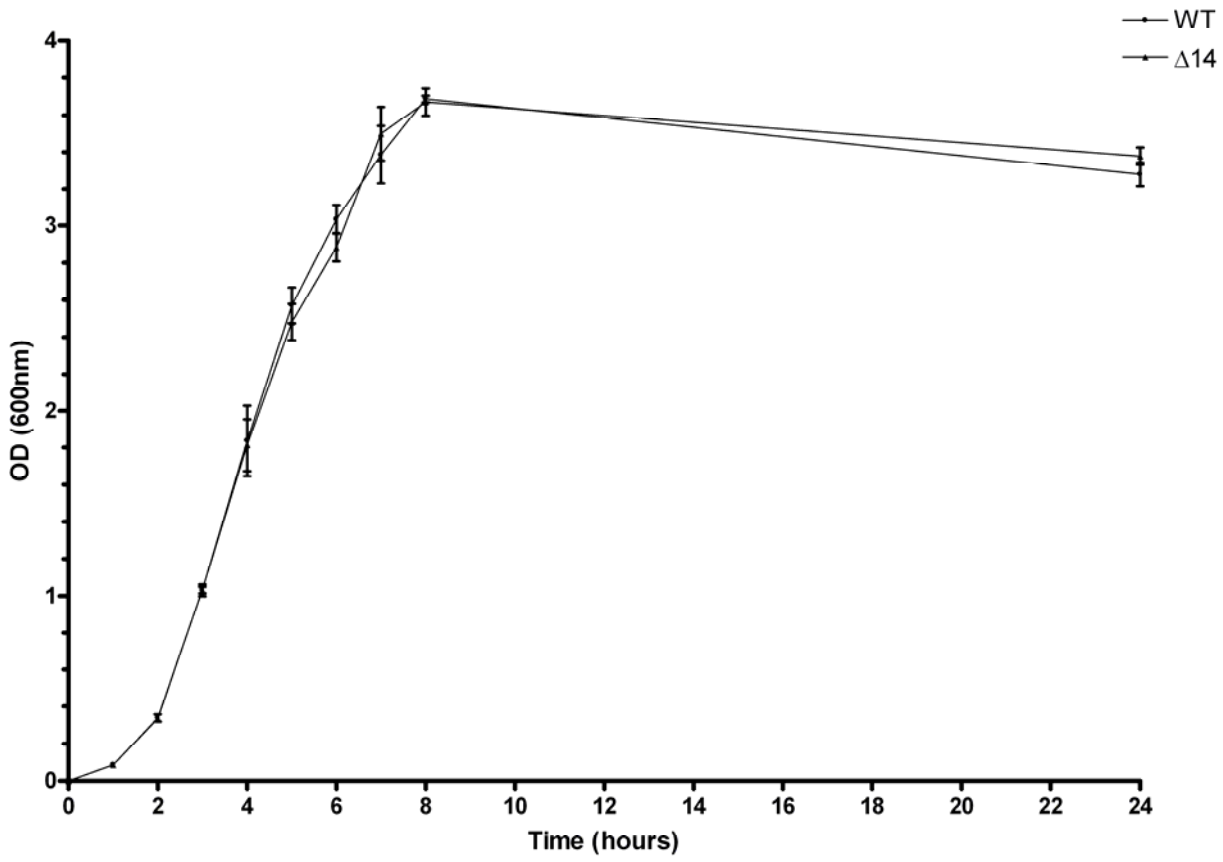

Figure 23. – Courbe de croissance de la souche afimbriaire par rapport à la souche sauvage.

Densité optique prise à longueur d'onde de 600nm de 0 à 24 heures pour la souche sauvage et la souche afimbriaire. Les courbes représentents au moins 3 réplicats. Les points représentent la moyenne ± SEM.

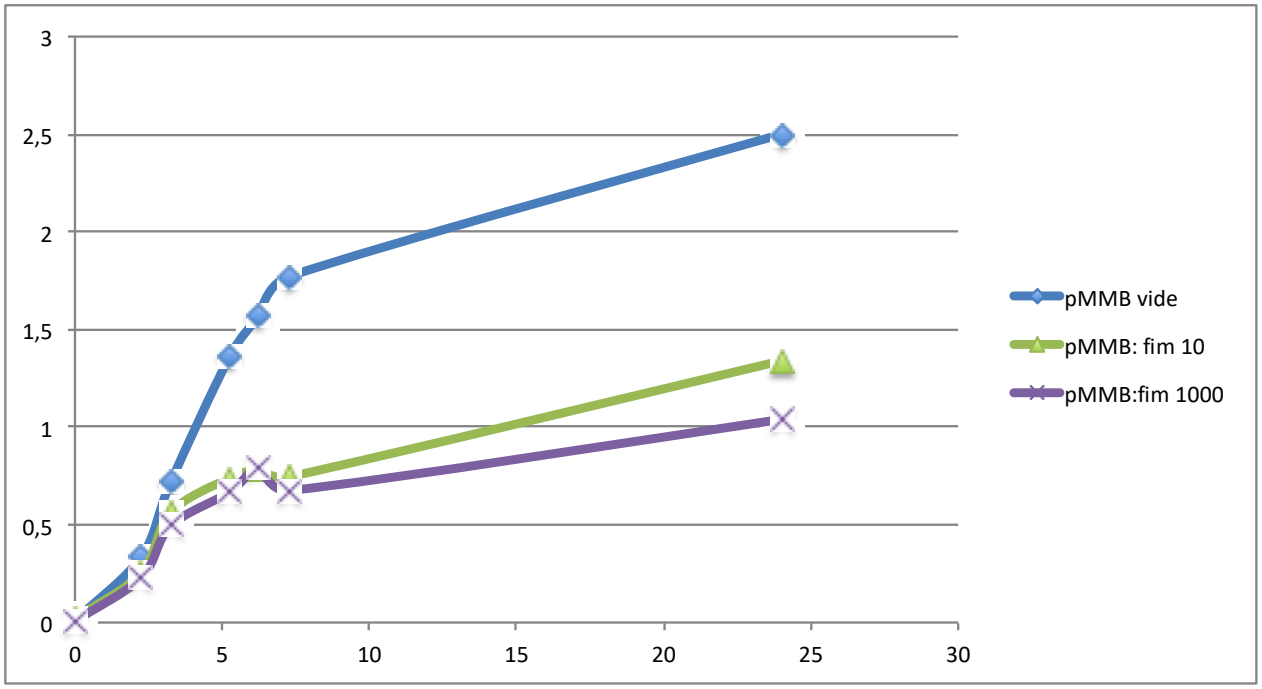

Figure 24. – Courbe de croissance de la souche afimbriaire avec vecteur inductible portant l'opéron *fim*

Densité optique prise à longueur d'onde de 600nm de 0 à 24 heures pour la souche afimbriaire portant le vecteur inductible vide ou avec opéron *fim*. Deux concentrations d'IPTG ont été ajoutées avec pMMB207c :*fim* (10 µM ou 1000 µM). Les courbes représentents au moins 3 réplicats. Les points représentent la moyenne ± SEM.

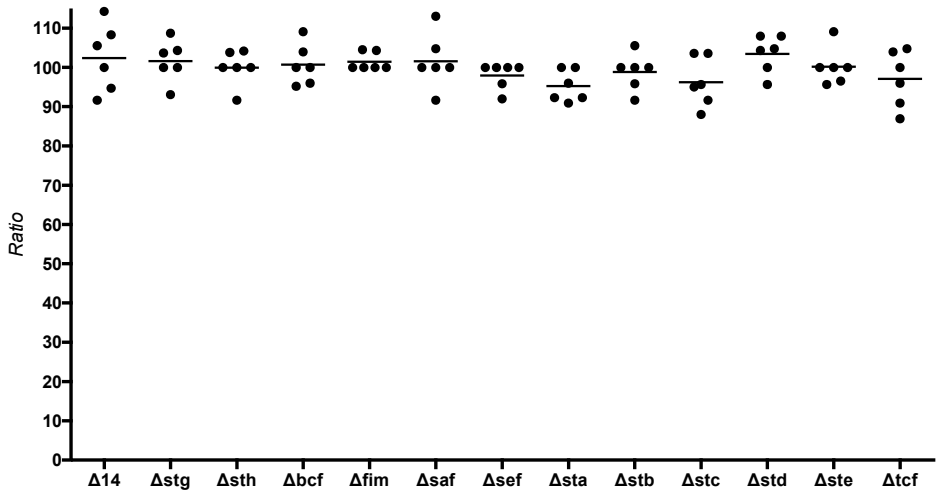

Figure 25. – Motilité des souches mutantes pour chacun des systèmes fimbriaires de *S.* Typhi

Le ratio de motilité de chaque souche est établi entre le diamètre de croissance de chaque souche mutante par rapport à la souche sauvage ayant cru sur la même gélose LB à 0,3% agar. Les résultats représentent au moins 6 réplicats. La ligne représente la moyenne des réplicats.

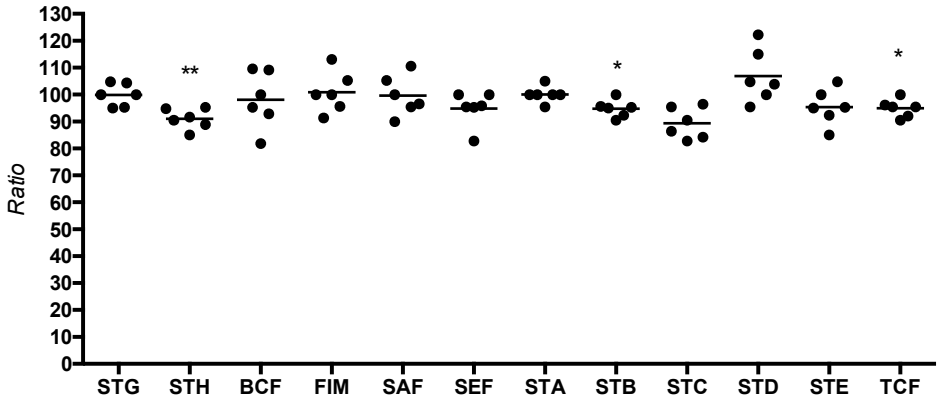

Figure 26. – Motilité des souches portant pWSK29 avec chacun des opérons fimbriaires natifs de *S.* Typhi

Le ratio de motilité de chaque souche est établi entre le diamètre de croissance de chaque souche portant pWSK29 avec opéron fimbriaire par rapport à la souche afimbriaire portant le plasmide vide ayant cru sur la même gélose LB à 0,3% agar. Les résultats représentent au moins 6 réplicats. La ligne représente la moyenne des réplicats.

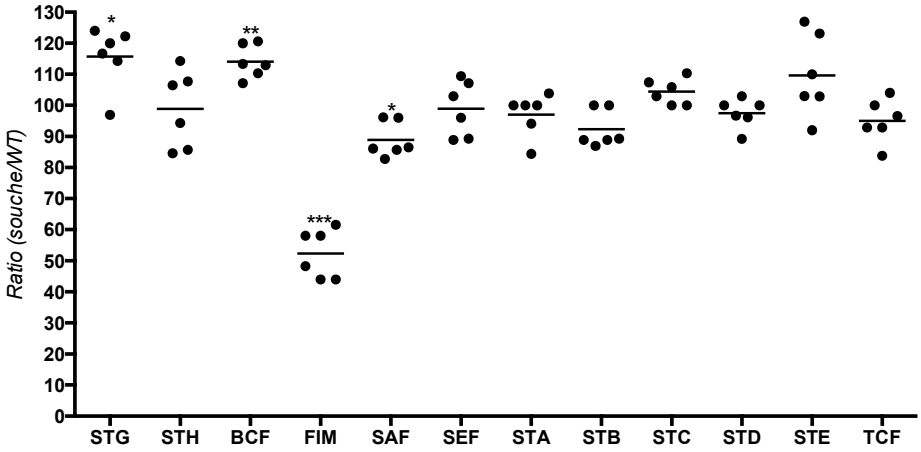

Figure 27. – Motilité des souches portant pMMB207c avec chacun des opérons fimbriaires de *S.* Typhi

Le ratio de motilité de chaque souche est établi entre le diamètre de croissance de chaque souche portant pMMB207c avec opéron fimbriaire par rapport à la souche afimbriaire portant le plasmide vide ayant cru sur la même gélose LB à 0,3% agar. Les résultats représentent au moins 6 réplicats. La ligne représente la moyenne des réplicats.

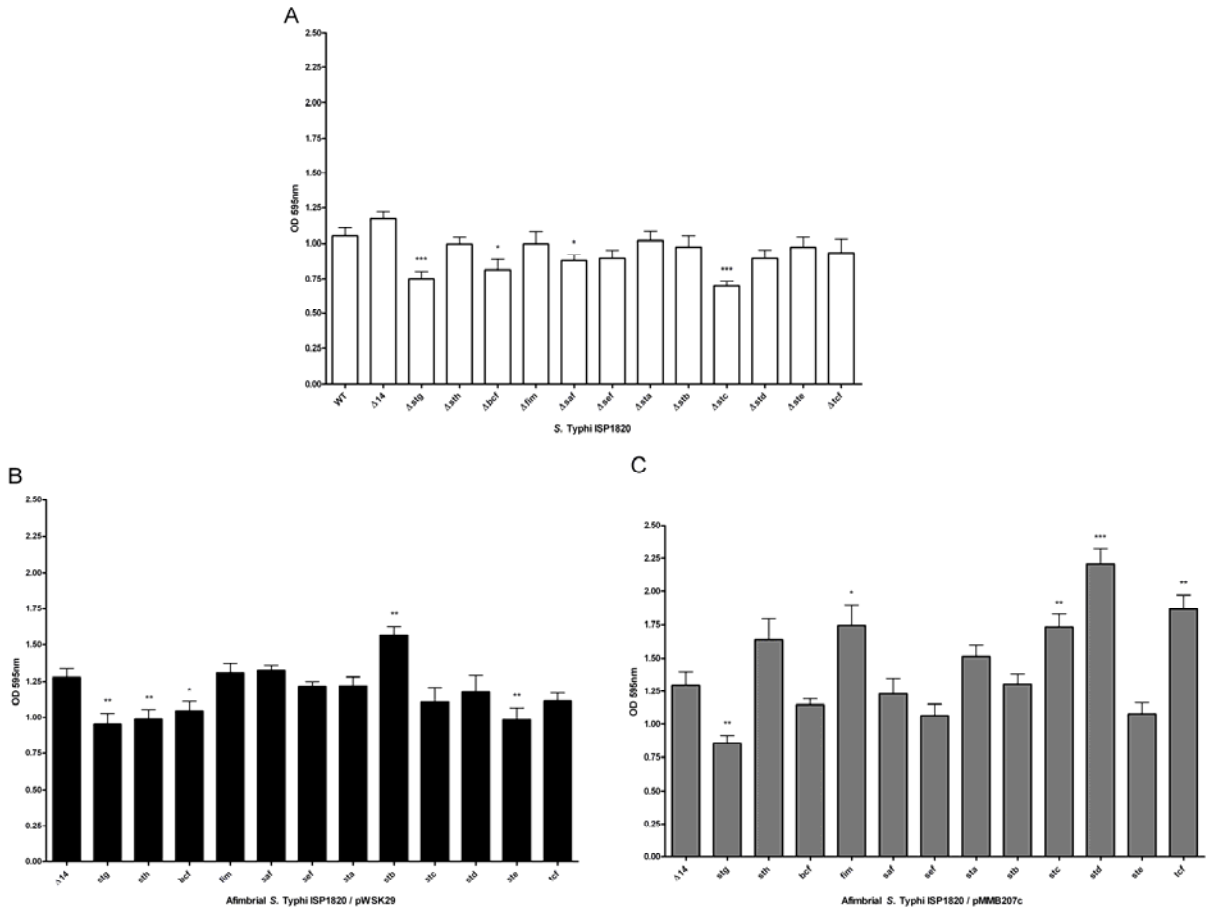

Figure 28. – Impact of fimbriae on biofilm formation.

Biofilm formation was performed with cholesterol-coated plate and bacteria were incubated statically for 72 hours in a bile-supplemented medium. Results are presented as the mean ± SEM of replicas. (A) Mutant strains with deletion of each fimbrial gene cluster were used for this assay. (B) Native promoter and operon was cloned on low-copy pWSK29 vector and transformed into afimbrial ISP1820. (C) Native operon was cloned under lactose-inducible promoter on pMMB207c vector and transformed into afimbrial ISP1820.

Tableau 13. – Détails des acronymes pour chacun des fimbriae de type chaperon-placier chez *S.* Typhi

Nom du fimbria	Détails de l'acronyme
Bcf	*Bovine colonization factor* (179)
Fim	Paralogue à opéron *fim* de *E. coli* (55, 180)
Saf	*Salmonella atypical fimbria* (181)
Sef	*Salmonella Enteritidis fimbria* (182)
Sta	Première identification chez *Salmonella* (ordre alphabétique) (55)
Stb	Première identification chez *Salmonella* (ordre alphabétique) (55)
Stc	Première identification chez *Salmonella* (ordre alphabétique) (55)
Std	Première identification chez *Salmonella* (ordre alphabétique) (55)
Ste	Première identification chez *Salmonella* (ordre alphabétique) (55)
Stg	Première identification chez *Salmonella* (ordre alphabétique) (55)
Sth	Première identification chez *Salmonella* (ordre alphabétique) (55)
Tcf	*Typhi colonization factor* (181)