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Peptide-utilizing carbon starvation gene *yjiY* is required for flagella-mediated infection caused by *Salmonella*

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Peptide metabolism forms an important part of the metabolic network of *Salmonella* and to acquire these peptides the pathogen possesses a number of peptide transporters. Whilst various peptide transporters known in *Salmonella* are well studied, very little is known about the carbon starvation (*cst*) genes *cstA* and *yjiY*, which are also predicted to be involved in peptide metabolism. We investigated the role of these genes in the metabolism and pathogenesis of *Salmonella*, and demonstrated for the first time, to the best of our knowledge, that *cst* genes actually participate in transport of specific peptides in *Salmonella*. Furthermore, we established that the carbon starvation gene *yjiY* affects the expression of flagella, leading to poor adhesion of the bacterium to host cells. In contrast to the previously reported role of *cstA* in virulence of *Salmonella* in *Caenorhabditis elegans*, we showed that *yjiY* is required for successful colonization of *Salmonella* in the mouse gut. Thus, *cst* genes not only contribute to the metabolism of *Salmonella*, but also influence its virulence.

INTRODUCTION

Salmonella enterica serovar Typhimurium is an enteric pathogen that infects a variety of hosts, including humans, cattle, poultry and rodents (Rabsch *et al.*, 2002). In humans, it is most frequently associated with diarrhoea, but can also lead to systemic invasive non-typhoidal disease (Feasey *et al.*, 2012), whereas in mouse it causes typhoid-like fever (Uzzau *et al.*, 2000). As the serovar Typhimurium brings about different outcomes of infection in different hosts, the diversity of such preferable niches makes its eradication a difficult task. This kind of adaptability exhibited by *S.* Typhimurium is attributed to its complex metabolic machinery assigned to utilize a plethora of nutrients available in the environment inhabited (Dandekar *et al.*, 2012; Steeb *et al.*, 2013).

As *Salmonella* encounters nutrition starvation inside or outside the host, it induces a starvation stress response to acquire alternate nutrients (Spector, 1998). Peptide transporters form an integral part of this system by mediating transport of specific peptides as nutrient source (Gibson *et al.*, 1984; Hiles *et al.*, 1987; Olson *et al.*, 1991). The importance of peptide transporters in virulence of several pathogens has been reported (McNab & Jenkinson, 1998; Moraes *et al.*, 2014; Podbielski & Leonard, 1998) and they have been considered as potential antibacterial targets (Garmory & Titball, 2004). Carbon starvation (*cst*) genes in *Salmonella* form one superfamily of putative peptide transporters (Krogh *et al.*, 2001), which derive their name from the fact that they become induced after the exhaustion of any carbon source in the medium (Schultz & Matin, 1991). Two homologous *cst* genes, *cstA* and *yjiY*, are conserved across *Enterobacteriaceae*, including *Salmonella* (Finn *et al.*, 2010). There is only one report where *cstA* has been shown to be required for virulence of *Salmonella* in *Caenorhabditis elegans*; however, to date no study has explored the mechanism behind this, most importantly in the mouse model of infection (Tenor *et al.*, 2004).

In *Escherichia coli*, the two peptide-utilizing *cst* genes *cstA* and *yjiY* are expressed under the control of cAMP at the onset of carbon starvation during the stationary phase of growth (Kraxenberger *et al.*, 2012; Schultz & Matin, 1991; Schultz *et al.*, 1988). Whilst *cstA* is negatively regulated by the global regulator CsrA (Dubey *et al.*, 2003), the expression of *yjiY* is positively regulated by the YehU/YehT two-component system (Kraxenberger *et al.*, 2012), indicating their independent modality of function in *E. coli*. YehU/YehT-mediated positive regulation of *cst*

Abbreviations: ABC, ATP-binding cassette; CFSE, 5, 6-carboxyfluorescein succinimidyl ester; q, quantitative; RT, real-time; TEM, transmission electron microscopy.

Nine supplementary figures are available with the online Supplementary Material.

genes has been reported in *Salmonella* (Wong *et al.*, 2013); however, the direct role of these genes in either metabolism or pathogenesis of *Salmonella* is not known. Bioinformatic analysis using the TMHMM protein topology prediction tool predicted 18 transmembrane helices in the CstA protein and 16 in YjiY (Krogh *et al.*, 2001), suggesting that these proteins might be spanning the membrane and transporting peptides inside the cells. The prediction of conserved domains using the National Center for Biotechnology Information database shows the lack of an ATP-binding domain in their predicted protein sequences, making them different from the ATP-binding cassette (ABC) transporter family of peptide transporters. Whilst many ABC transporters for peptides are well characterized in the pathogen *Salmonella* (Gibson *et al.*, 1984; Hiles *et al.*, 1987; Olson *et al.*, 1991), the function of *cst* genes remains unaddressed, especially in mammalian host systems.

In this study, we established that *cst* genes mediate transport of specific peptides and thereby contribute to growth of *S.* Typhimurium whilst working independently of each other. We have also shown that *yjiY* affects the formation of flagella in *S.* Typhimurium and thereby establishment of infection in the mammalian host. Whilst the Δ*yjiY* strain displayed reduced colonization of mouse intestine, the Δ*cstA* strain was unable to colonize the gut of *C. elegans*. Thus, the two homologous *cst* genes known in *Salmonella* function independently of each other during the life cycle of the pathogen.

METHODS

Bacterial strains and growth conditions. All bacterial strains used in this study are listed in Table 1. *S.* Typhimurium ATCC 14028, a gift from Professor Michael Hensel, was used as the parental WT strain to generate the knockout strains Δ*cstA*, Δ*yjiY*, Δ*fliC* and the complement strain Δ*yjiY*-pQE60-*yjiY*. All strains were maintained in Lennox broth

Table 1. Strains and plasmids

Strain or plasmid	Description	Reference
Strain		
S. Typhimurium ATCC 14028	WT	Kind gift from Professor Michael Hensel (Max von Pettenkofer-Institute for Hygiene and Medizinische Mikrobiologie, Germany)
Δ*cstA*	Kan^r	This study
Δ*yjiY*	Kan^r	This study
Δ*fliC*	Chl^r	Laboratory work
Δ*yjiY*-pQE60-*yjiY*	Complement strain for Δ*yjiY* expressing *yjiY* under the promoter of T5 present in the plasmid pQE60	Laboratory work
Δ*invC*	No antibiotic resistance cassette	Kind gift from Professor Michael Hensel (Max von Pettenkofer-Institute for Hygiene and Medizinische Mikrobiologie, Germany)
Plasmid		
pKD4	3.2 kb, Kan^r, derived from pANTSγ and pCP15	Datsenko & Wanner (2000)
pKD3	2.8 kb, Chl^r, derived from pANTSγ and pSC140	Datsenko & Wanner (2000)
pKD46	6.3 kb, derived from pINT-ts and pBAD18, Amp^r	Datsenko & Wanner (2000)
pQE60	3.4 kb, contains T5 promoter, T5 transcription start site and ColE1 origin of replication, Amp^r	Das *et al.* (2009)
pTrc-*fliC*	4.5 kb, *trc* expression vector containing *fliC* of *S.* Typhimurium, *lacI*^{q}, Amp^r	Amann *et al.* (1988); modified in laboratory
pFPV-mCherry	5.3 kb, Amp^r	Drecktrah *et al.* (2008)

(LB) under shaking conditions at 170 r.p.m. or on agar plates at 37 °C with respective antibiotics. Kanamycin and ampicillin were added at 50 μg ml^{-1}, and chloramphenicol at 10 μg ml^{-1}. For growth curve experiments, overnight culture in LB was subcultured (1 : 100) in LB or M9 medium with 1 % casein (w/v) and incubated at 37 °C under shaking conditions. OD_{600} was measured at different time intervals.

Generation of knockout strains. All knockout strains were generated using a one-step inactivation method based on λ recombinase, encoded in plasmid pKD46, mediated recombination (Datsenko & Wanner, 2000). The target gene was replaced with either a kanamycin or chloramphenicol resistance gene, amplified from template plasmids pKD4 and pKD3, respectively. The plasmids used are described in Table 1. The knockout and confirmatory primers are listed in Table 2.

Phenotype microarray analysis. Phenotype microarray analysis of peptide substrates was performed as per the manufacturer's protocol provided by Biolog (Bochner, 2009). The WT and isogenic *cst* mutant strains were grown overnight on Biolog Universal Growth (BUG) agar at 37 °C. Cells were taken with sterile cotton swabs and added to 16 ml IF-0 inoculation fluid until the transmittance reached 42 %. This was diluted in IF-0 + Dye-A solution to obtain 85 % transmittance. Sodium pyruvate was added as a carbon source at 20 mM. An aliquot of 100 μl prepared inoculum was added to each well of

Table 2. Oligonucleotides

Primer	Sequence (5′→3′)
Knockout primers	
cstA KO fwd	CAAAATGTAACATCTCTCTGGAACACCCAAACGGACAACAACTGTGTAGGC TGGAGCTGCTTC
cstA KO rev	GCCCTCTCCTTATTCTGGAGAGGGCTATTGATGTAAAAAGACATATGAATAT CCTCCTTAG
yjiY KO fwd	GGCCTACACTAGCCGGGGTCGCTGTGCGCTACCCGGCATCAGTTGTGTAGG CTGGAGCTGCTTC
yjiY KO rev	CCACCCAGCTTCGGCTGATAATTAAACTTACAACCAGGTTTTACTGGTTTCT GTTAACACTGAAG
Confirmatory primers	
cstA KO CON fwd	GGTAACAATCCGGTTTTATC
cstA KO CON rev	CGAACATCGTTGTCACCTG
yjiY KO CON fwd	TCTGCCGTCCTGTTAATGAA
yjiY KO CON rev	CATAGCAGTCAATCTACCTC
Cloning primers	
yjiY pQE60 cloning fwd	ATGCGGATCCATGGATACGAAAAAGATATTC
yjiY pQE60 cloning rev	ATGCAAGCTTTTAGTGGTGTGAAGAGA
qRT-PCR primers	
RT *fimA* fwd	GGCTGATCCTACTCCGGTGA
RT *fimA* rev	TTACCAATCGCCGTAAAGC
RT *lpfA* fwd	GGTGGATTTACCGGTGTTCA
RT *lpfA* rev	GGTTTCTGTTAACACTGAAG
RT *siiE* fwd	ACGACGAAATAAGAAGGAAG
RT *siiE* rev	TTCTGTACTTCAAACGCTTC
RT *csgA* fwd	GGATTCCACGTTGAGCATT
RT *csgA* rev	TTCCACTGGTCGATGGTG
RT *fliC* fwd	GTATAAGACATCTTAACAAC
RT *fliC* rev	GAACGGTGAGGTGACTCTTG
RT *flhD* fwd	TGCTGTAAGTCATCGACGCG
RT *flhD* rev	AAAGCATCTGCGATGTTCCG
RT *fljB* fwd	ACGAATGGTACGGCTTCTGTA
RT *fljB* rev	TACCGTCGATAGTAACGACTT
RT *cheM* fwd	AGCAACAAAGCGAACTCACG
RT *cheM* rev	GCTGCTCTGCTGGTTAGAAG
RT *fliA* fwd	GTTCCTTGCAAAGCGTCATA
RT *fliA* rev	TACCGCTGAAGGTGTAATGG
RT *fliM* fwd	CCGTTACGTCTTTCCCAGAT
RT *fliM* rev	ATTTGGGCTGTTCCTCATTC
RT *cstA* fwd	GGCTATCTGCCGGGGATGATC
RT *cstA* rev	TCGCGCCCATCTCCTCTTTAAC
RT *yjiY* fwd	CGCCTCTCTTGGCGAGATGATC
RT *yjiY* rev	CGGCACCGTGGAGCAGACG

PM6, PM7 and PM8 plates containing di- and tripeptides. The plates were incubated at 37 °C in an OmniLog incubator for 48 h. As the bacterial cells utilize the peptides and respire, they reduce the indicator dye tetrazolium violet to produce a purple colour. The colour intensities were recorded at intervals of 15 min and the corresponding kinetic respiration data generated were analysed by OmniLog-PM software. Comparison of the values of mutant strains with respect to that of WT was represented by colour-coded images.

Peptide uptake assay. The dipeptides were synthesized by solid-phase peptide synthesis using standard 9-fluorenylmethoxycarbonyl-based chemistry (Chatterjee *et al.*, 2012). The C-terminal amino acid was loaded onto 2-Cl trityl chloride polystyrene resin using 1.5 eq. amino acid and 3 eq. *N*,*N*-diisopropylethylamine in dry dichloromethane overnight. The loading capacity was calculated and used for further coupling using diisopropylcarbodimide (2.5 eq.), 1-hydroxybenzotriazole (2.5 eq.) and the amino acid (2.5 eq.) for 2 h. 5,6-Carboxyfluorescein succinimidyl ester (CFSE) was attached to the N terminus of the peptide using 5,6-carboxyfluorescein, 1-hydroxy-7-azabenzotriazole, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate, 3 eq. each, with 5 eq. *N*,*N*-diisopropylethylamine overnight. Global cleavage was performed with 95 % trifluoroacetic acid/2.5 % triisopropylsilyl/2.5 % H_2O followed by precipitation in chilled ether. Purification of the peptide was carried out using reverse-phase HPLC, with a C18 capcell-pack semiprep column on a 15–75 % acetonitrile/water gradient. The purified sample was lyophilized and dissolved in MilliQ to obtain a stock solution of 1 mM. An aliquot of 1 ml overnight bacterial culture was pelleted and washed twice with sterile PBS. Then, 10^8 c.f.u. were added in 1 ml PBS containing 50 μM dipeptide. After incubation at 37 °C for 48 h, cells were pelleted down and washed with PBS. These cells were imaged using CLSM (Zeiss LSM 710) with a × 63 oil immersion objective. Images were analysed using ZEN 2009 software.

Growth in the presence of the dipeptide Lys–Val. The complete minimal medium was prepared with similar a composition as used for phenotype microarray analysis except for the dye. The medium contained 100 mM NaCl, 30 mM triethanolamine/HCl (pH 7.1), 25 mM sodium pyruvate, 5.0 mM NH_4Cl, 2.0 mM NaH_2PO_4, 0.25 mM Na_2SO_4, 0.05 mM $MgCl_2$, 1.0 mM KCl and 1.0 μM ferric chloride, and was filter-sterilized. Overnight cultures of all strains in LB were pelleted down at 2500 ***g*** for 5 min and washed twice in the minimal medium. The pellet was finally resuspended in minimal medium without the dipeptide. From this, 10^6 c.f.u. were inoculated in 1 ml minimal medium containing the dipeptide Lys–Val at the concentration of 50 μM and incubated at 37 °C under shaking condition. Aliquots were taken at different time points and plated on LB agar to obtain c.f.u. values for plotting the growth curve.

Microarray analysis. *S.* Typhimurium 14028 and its derivatives Δ*cstA* and Δ*yjiY* were grown in LB to late exponential phase (OD_{600} 1.5). RNA was isolated from equal volumes of cultures of two biological replicates for each strain by TRIzol-based phase separation method. RNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer, and the integrity of total RNA was verified on an Agilent 2100 Bioanalyzer using the RNA 6000 Nano Lab Chip (Agilent Technologies). The samples were labelled using an Agilent Quick-Amp labelling kit. Samples of 500 ng each total RNA were reverse transcribed at 40 °C using WT primer with a T7 polymerase promoter and converted to double-stranded cDNA. These were used as template for cRNA generation by *in vitro* transcription at 40 °C whilst the dye Cy3 CTP (Agilent) was incorporated during this step. Labelled cRNA was cleaned up using Qiagen RNeasy columns, and quality was assessed for yields and specific activity using a NanoDrop ND-1000. Samples of 1000 ng labelled cRNA sample were fragmented at 60 °C using a Gene Expression Hybridization kit (Agilent Technologies) and hybridized onto Genotypic designed Custom *Salmonella* GXP, 8 × 15K (AMADID 046639) arrays. The hybridized slides were scanned using an Agilent Microarray Scanner (Agilent Technologies). Data extraction from images was done using Agilent Feature Extraction software and analysed using GeneSpring GX software from Agilent. Differentially regulated genes were clustered using hierarchical clustering based on the Pearson coefficient correlation algorithm to identify significant gene expression patterns. Genes were classified into functional category and pathways based on gene ontology.

Quantitative real-time (qRT)-PCR. An aliquot of 1 ml culture in LB from either exponential phase (OD_{600} 1.0), late exponential phase (OD_{600} 1.5) or stationary phase (OD_{600} 2.0) was lysed with lysozyme and TRIzol reagent (Sigma). RNA was isolated by a phase separation method using chloroform. A sample of 2 μg RNA was used to synthesize cDNA by Bioline MuMLV reverse transcriptase (Tetro). qRT-PCR was carried out using a SYBR Green RT-PCR kit (Kapa Biosystems) in a 7900HT real-time PCR detection system (ABI). Relative expression with respect to expression in WT, after normalizing with the values obtained for the reference gene 16S rRNA, was plotted as fold change.

Invasion assay. All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) at 37 °C and 5 % CO_2. Caco-2 cells were polarized by incubating for 14 days whilst changing the medium every third day. Monolayers of the cell lines HeLa, Intestine-407 and Caco-2 polarized cells were infected with bacteria grown up to late exponential phase in LB (OD_{600} 1.5), whereas RAW 264.7 cells were infected with overnight culture (OD_{600} 2.0). Each cell line was infected at m.o.i. 10. Bacterial attachment to host cells was enhanced by centrifuging at 200 ***g*** for 10 min. After 25 min of infection, cells were treated with gentamicin (100 μg ml^{-1} in DMEM) for 1 h to remove extracellular bacteria. Then, 0.1 % Triton X-100 (v/v in PBS) was used to lyse the cells and the lysate was plated on LB agar plates. Per cent invasion was calculated with respect to the bacterial inoculum used for infection.

Adhesion assay. For adhesion assay by CLSM, we adapted a method described previously (Gerlach *et al.*, 2007). Infection of cell lines was carried out as described earlier at m.o.i. 50. After allowing bacteria to attach to the cells for 25 min (15 min for RAW 264.7 cells), non-adhered bacteria were removed by washing with PBS and cells were fixed with 3.5 % paraformaldehyde (w/v in PBS) for 15 min at room temperature. Bacteria were stained with rabbit anti-*Salmonella* O-antigen antibody (HyTest) and subsequently with Alexa Fluor 488 fluorophore conjugated anti-rabbit antibody (Jackson ImmunoResearch). Antibodies were diluted in PBS containing 2 % BSA (w/v) without adding saponin, so that only the adhered bacteria were stained and not the internalized bacteria. Images were obtained by CLSM (Zeiss LSM 710) using a × 63 oil immersion objective. The number of bacteria adhered per cell was calculated by dividing the total number of bacteria adhered by the total number of host cells, counted with the help of ZEN 2009 software, for each image.

Transmission electron microscopy (TEM). Between 2 and 5 μl overnight culture grown in 5 ml LB broth was placed on a TEM copper grid (Electron Microscopy Sciences) and cells were allowed to settle for 1 min. The excess liquid was soaked with a tissue paper from the edge of the grid. Between 2 and 5 μl PBS was placed on the grid for 1 min to wash the cells. Finally, the cells were stained with 1 % uranyl acetate in water (w/v) for 1 min and air-dried. The grids were then taken for imaging using an ICON transmission electron microscope.

Motility assays. Swim and swarm agar plates were prepared with 0.3 and 0.5 % agar (w/v), respectively. Other components included 0.5 %

glucose, 0.5 % NaCl, 0.5 % yeast extract and 1 % casein enzyme hydrolysate. Overnight cultures grown in LB were spotted on the swim or swarm agar plates and incubated at 37 °C for 4–6 h. The images of the plates were obtained by using the Bio-Rad gel documentation system.

Infection of animal models. BALB/c mice (6–8 weeks old) housed at the Central Animal Facility of the Indian Institute of Science were used for all the *in vivo* experiments. All procedures with animals were carried out in accordance with the institutional rules for animal experimentation. All animal experiments were approved by the Institutional Animal Ethics Committee and the National Animal Care Guidelines were strictly followed. In the colitis model, mice were treated with 5 mg streptomycin *per os* 24 h prior to infection. Each mouse was infected orally with 10^8 c.f.u. overnight culture in LB (OD_{600} 2.5). At 24 h post-infection, mice were sacrificed and caecal content was plated on *Salmonella–Shigella* (SS) agar. For the *in vivo* invasion assay, mice were gavaged with 10^8 c.f.u. from late-exponential-phase culture (OD_{600} 1.5) grown in LB. After 5 h of infection, mice were sacrificed and Peyer's patches were isolated. The bacterial burden on Peyer's patches was evaluated by homogenizing them with sterile glass beads (1 mm diameter) in PBS and plating on SS agar. For the systemic infection model, mice were orally gavaged with 10^7 c.f.u. overnight culture grown in LB. At 5 days post-infection, mice were sacrificed, and organ burden for liver and spleen was evaluated for each mouse by plating the homogenate of these organs on SS agar. For infection of *C. elegans*, 10^8 c.f.u. bacterial strains harbouring pFPV-mCherry plasmid were seeded on nematode growth medium agar (3 g NaCl l^{-1}, 2.5 g peptone l^{-1}, 17 g agar l^{-1}, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 μg cholesterol ml^{-1}, 400 mM KPO_4, 50 μg streptomycin ml^{-1}). After formation of a bacterial lawn, ~100 worms were added to each plate. The worms were allowed to feed on the bacterial lawn for 12 h and then transferred to 1.5 ml tubes by washing with MilliQ water. These worms were allowed to settle by centrifuging at 100 ***g*** for 5 min and given two washes with MilliQ to remove non-ingested bacteria. Worms were fixed with levamazole and imaged by CLSM (Zeiss LSM 710) using a ×10 objective lens.

Statistical analysis. Statistical significance was calculated either by one-way ANOVA or by Student's *t*-test using GraphPad Prism 5.0 software. The type of statistical test used for each dataset is described individually. Significance in the figures is represented as: $*P<0.05$; $**P<0.005$; $***P<0.001$.

RESULTS

cst genes aid in peptide uptake by *Salmonella*

The two carbon starvation genes *cstA* and *yjiY* are highly similar with 75 % identity in their nucleotide sequences. However, they are located at different locations in the genome of *Salmonella* (Fig. 1). In *E. coli*, *cstA* is predicted to code for an integral membrane protein that facilitates peptide uptake (Schultz & Matin, 1991). Based on the homology between *cst* genes of *E. coli* and *Salmonella* (87 % identity), we asked the question if *cst* genes are required for peptide utilization in *Salmonella* as well? Biolog phenotype microarray analysis, performed on various peptide nutrient sources, showed compromised utilization of several peptides by both the *cst* mutants (shown in red in Fig. 2). Interestingly, the Δ*yjiY* strain could grow better in the presence of certain peptides than the WT strain (shown in green in Fig. 2b), which was not observed

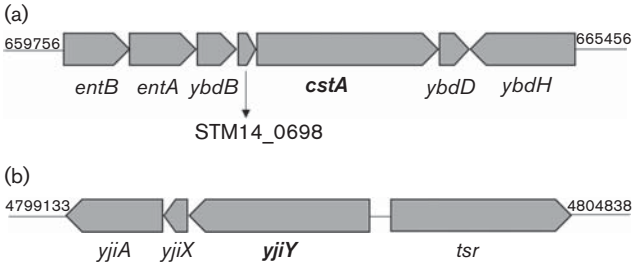

Fig. 1. Genomic organization of *cst* genes: location of (a) *cstA* and (b) *yjiY* in the genome of *S.* Typhimurium strain 14028 together with the neighbouring genes.

in the case of the Δ*cstA* strain. Ten peptides for each strain showing the maximum difference from WT are listed in Table 3. Most of the peptides listed contain lysine residues, indicating that transporters encoded by *cst* genes could be the major transporters for peptides of lysine. The difference observed with respect to the WT was more prominent in the case of Δ*yjiY* than Δ*cstA* (Table 3), suggesting a more significant role of *yjiY* in peptide utilization than *cstA*.

To determine whether the reduced utilization of peptides by Δ*yjiY* is at the level of transport or metabolism, a fluorescent peptide uptake assay was designed based on the principle that the absence of the respective transport system will result in poor uptake of fluorescently labelled peptides and hence reduced numbers of fluorescent cells. Even if the peptides are digested to release amino acids, the cells will still remain fluorescent as the fluorophore used here, CFSE, can remain stable *in vivo* for several days (Graziano *et al.*, 1998). As a result, only uptake of the dipeptide will be scored and not its metabolism. The dipeptide Lys–Val was selected for chemical synthesis, as this peptide gave the highest negative difference between the respiration kinetics of WT and Δ*yjiY* in the phenotype microarray analysis (Table 3). The dipeptide was tagged with CFSE at the N terminus and provided to WT, Δ*yjiY* and complement strain Δ*yjiY*-pQE60-*yjiY* for 48 h at 37 °C in PBS in the absence of any other nutrient sources. The presence of fluorescent bacterial cells was checked by CLSM and after quantification it was observed that the percentage of fluorescent Δ*yjiY* cells was significantly lower than that of WT (Fig. 3). The complement strain Δ*yjiY*-pQE60-*yjiY* could rescue the phenotype ensuring that the transport of this peptide is dependent on *yjiY*. The expression of *yjiY* in the complement strain was validated by amplifying the cDNA (Fig. S1, available in the online Supplementary Material). We noticed that a maximum 2 % of the total population of cells could take the peptides, probably due to limiting amounts of peptide (50 μM) in the medium. The dipeptide Ser–Lys was selected outside of the phenotype microarray and used as a negative control. The rationale behind selecting this dipeptide was that in contrast to most of the dipeptides listed that possess lysine at the N terminus, we changed the orientation of this

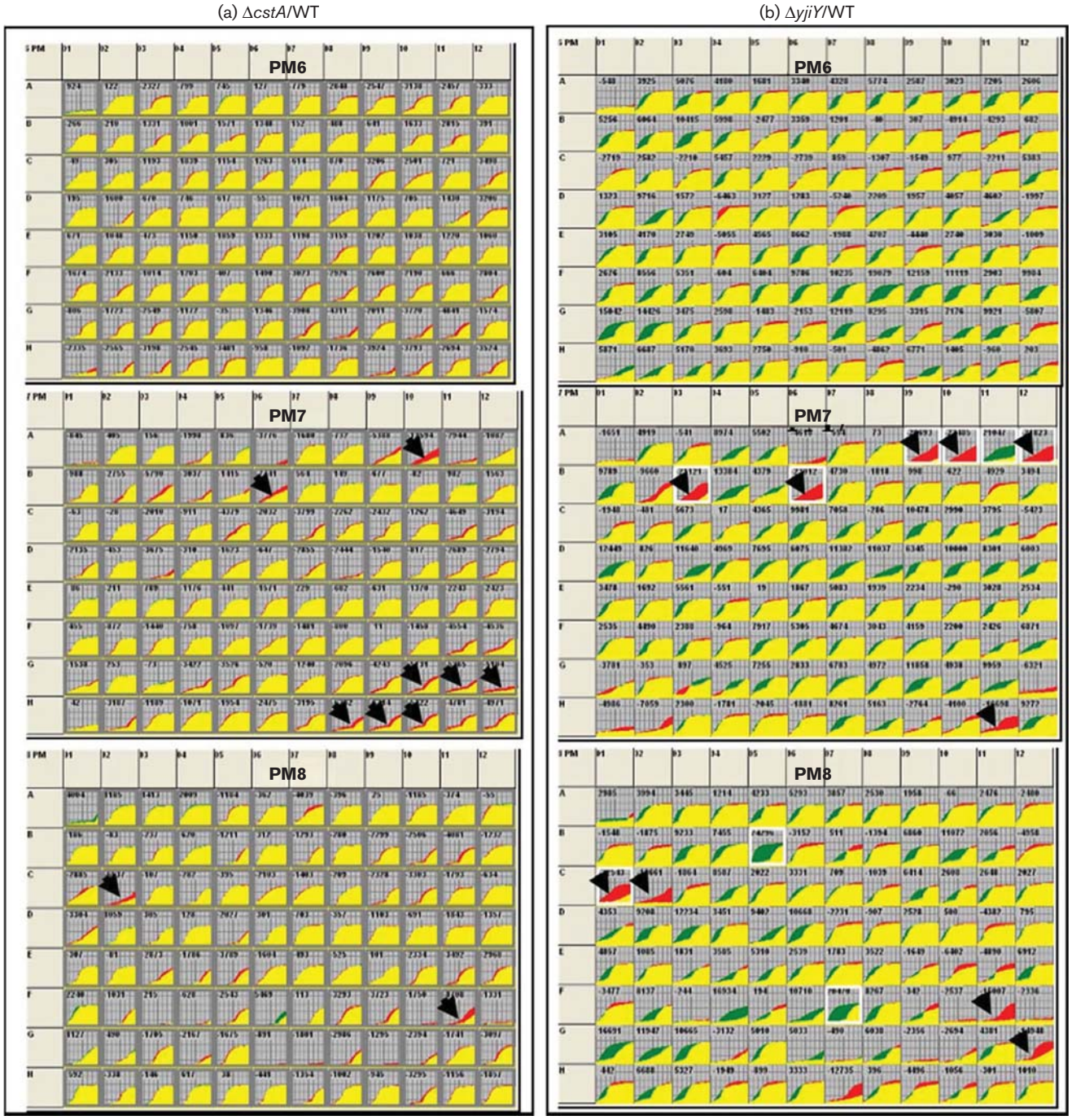

Fig. 2. Phenotype microarray analysis of peptide substrates. All strains were grown on Biolog phenotype microarray plates PM6, PM7 and PM8, containing dipeptides and tripeptides, for 48 h. The respiration kinetics obtained were plotted for the (a) Δ*cstA* and (b) Δ*yjiY* strains against WT. Red shows reduced (phenotype lost), green shows increased (phenotype gained) and yellow shows equivalent level of respiration with respect to WT. Black arrowheads point to the peptides showing maximum reduction in respiration of the knockout strain compared with WT.

residue to the C terminus. Serine, however, was selected for the N terminus because this amino acid was not present in any of the peptides listed. There was no significant change observed in the per cent uptake of Δ*yjiY* compared with WT for the dipeptide Ser–Lys. This verifies that *yjiY* is involved in transporting specific peptides in *Salmonella*.

Table 3. List of 10 peptides with the highest negative differences in respiration kinetics of each knockout strain with respect to the WT strain

Each value was obtained by subtracting the area of the curve (AUC) of the knockout strain from that of the WT strain, calculated by Omni-Log software and shown in the images.

Peptides not utilized by Δ*cstA*	Difference from WT (AUC)	Peptides not utilized by Δ*yjiY*	Difference from WT (AUC)
Lys–Leu	−12594	Lys–Val	−23012
D-Leu–Gly	−8708	Lys–Gly	−22543
Lys–Val	−7741	Lys–Leu	−22485
Lys–Met	−6637	Lys–Phe	−21823
Val–Ile	−6282	Lys–Thr	−21121
Val–Leu	−6214	Lys–Ile	−20693
Tyr–Leu	−5731	Val–Val	−16698
Val–Tyr	−5322	D-Leu–Gly	−15007
Tyr–Lys	−5265	D-Ala–Gly–Gly	−14948
Tyr–Phe	−5104	Lys–Met	−13661

However, growth of both the *cst* mutants was not affected in peptide-rich media (Fig. S2A). This could be due to the presence of various other peptides in the media which could be transported by alternate peptide transporters (Olson *et al.*, 1991). The upregulation of both the genes in the stationary phase in LB (Fig. S2B) confirms their role in peptide metabolism in *Salmonella* during nutrient starvation. To verify that the dipeptides taken up contributed to the growth of the cell, a growth curve experiment was carried out in the presence of the dipeptide Lys–Val as a nutrient source. The growth conditions were similar to those used for the phenotype microarray analysis. The mutant Δ*yjiY* indeed showed poor growth in the presence of the dipeptide compared with the WT and complement strain (Fig. S3).

yjiY, but not *cstA*, is required for invasion of host cells

Peptides being transported inside bacteria not only serve as a source of energy, but can also have additional functions ranging from functionality of membrane proteins to virulence of a pathogen (Alix & Blanc-Potard, 2009; Detmers *et al.*, 2001). In Gram-positive pathogens, peptides act as signal peptides which regulate quorum sensing and virulence (Cundell *et al.*, 1995; Moraes *et al.*, 2014; Samen *et al.*, 2004; Slamti & Lereclus, 2002). However, involvement of peptide transporters in the pathogenesis of Gram-negative pathogens is not well understood. The importance of one of the *cst* genes, *cstA*, in *Salmonella* virulence was reported previously in *C. elegans* (Tenor *et al.*, 2004). Thus, it is intriguing to unravel the role of *cst* genes in *Salmonella* pathogenesis. We carried out uptake assays on the macrophage cell line RAW 264.7 and invasion assays on epithelial cell lines of polarized Caco-2 cells, which mimic the brush border of the human gut (Peterson & Mooseker, 1992), Intestine-407 and HeLa. The Δ*yjiY* strain showed reduced percentage of invasion in all these cell types, whereas the Δ*cstA* strain behaved like the WT strain (Fig. 4). The defective invasion of Δ*yjiY* was rescued by the complement strain (Fig. S4). Whilst the invasion of epithelial cells depends on the expression of the SPI1 type 3 secretion system needle (Raffatellu *et al.*, 2005), the mode of entry of *Salmonella* in macrophages is independent of the function of SPI1 (Aderem & Underhill, 1999), ruling out the role of SPI1 in *yjiY*-dependent invasion of host cells.

Invasion attenuation of Δ*yjiY* is due to reduced adhesion

As adhesion is a prerequisite for entry of *Salmonella* inside host cells (Bäumler *et al.*, 1996; Dibb-Fuller *et al.*, 1999), we set out to assess the adhering capacity of the *cst* mutants by *in vitro* adhesion assays. The distribution of adhered bacteria on host cells, visualized by CLSM, showed that the Δ*yjiY* strain adhered poorly on macrophage cell line RAW 264.7 (Fig. 5), epithelial cell line HeLa (Fig. 6) and polarized Caco-2 cells (Fig. S5), whereas the Δ*cstA* strain had an equivalent level of adherence compared with the WT strain (Fig. S5). In fact, the invasion-deficient strain Δ*invC* (Akeda & Galán, 2004) could adhere well to macrophages as well as epithelial cells (Fig. S6). This clarifies that the reduced entry of Δ*yjiY* in host cells is due to defective adhesion and independent of an intact invasion machinery of *Salmonella*. Also, *yjiY*-dependent adhesion of *Salmonella* does not depend on the type of host cells, which is in accordance with the results obtained for invasion assays. This confirms that adhesion of *Salmonella* to host cells depends on the presence of *yjiY* to help in successful internalization of the pathogen in the target cell.

Flagella synthesis in *Salmonella* depends on *yjiY*

To understand the basis behind *yjiY*-dependent adhesion of *Salmonella* to the host cells, transcriptomes of the WT and *cst* mutant strains were compared by microarray (GEO accession number GSE6731). The results showed that transcription of the genes belonging to the major fimbrial adhesion operons in *Salmonella* (Weening *et al.*, 2005) were unaffected. This was validated by qRT-PCR for a few major fimbrial adhesion-associated genes: *fimA* (type I fimbriae), *lpfA* (long polar fimbriae) and *csgA* (curli) (Fig. 7). As *yjiY*-dependent adhesion was found to be independent of host cell type and these adhesins function in a host cell-specific manner (Bäumler *et al.*, 1996; Weening *et al.*, 2005), we ruled out the role of these adhesins in this phenotype. We next looked for the expression of certain non-fimbrial adhesins like *siiE* (Gerlach *et al.*, 2007) and flagella biosynthesis-related genes (Dibb-Fuller *et al.*, 1999; Haiko & Westerlund-Wikström, 2013). Whilst the expression of *siiE* was not significantly altered, *fliC* was found to be severely downregulated (Fig. 7), which agreed with the microarray data. As flagellum is an

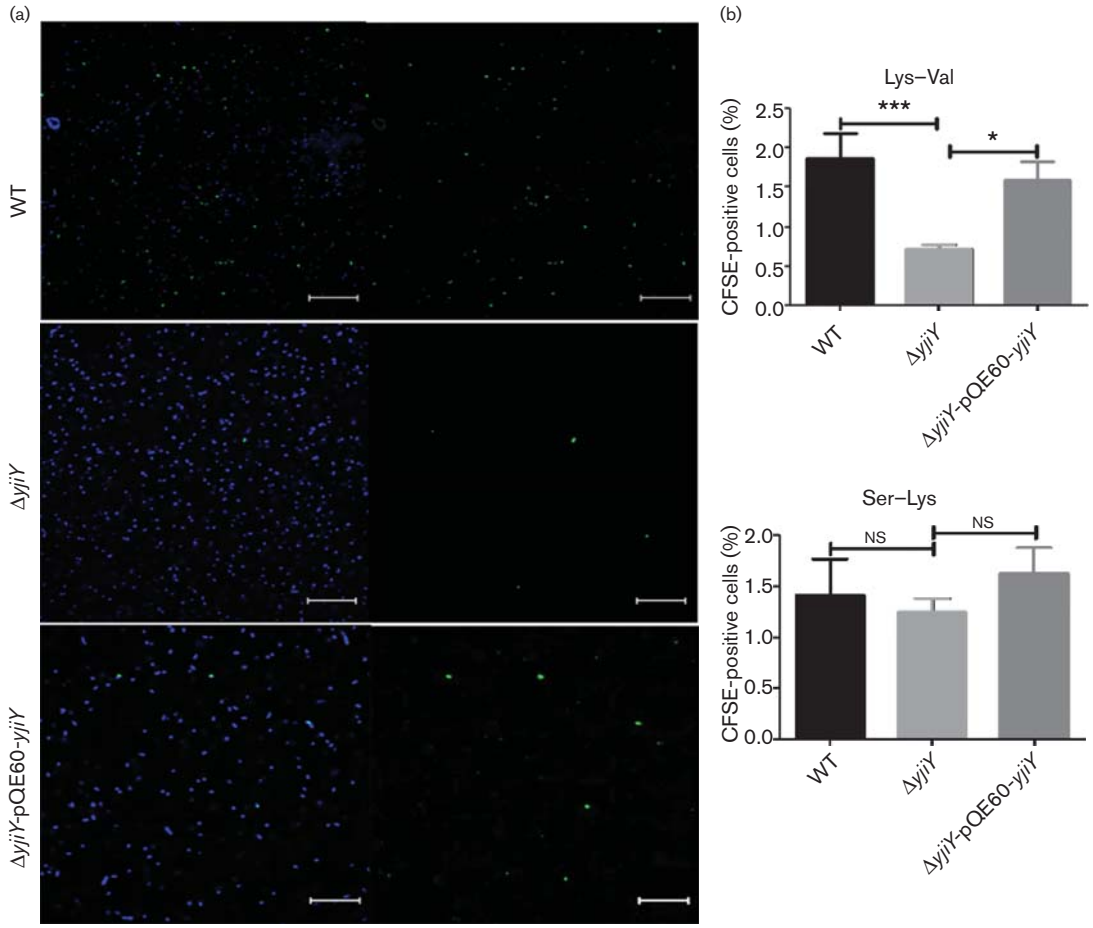

Fig. 3. (a) Fluorescent peptide uptake assay. The WT, Δ*yjiY* and Δ*yjiY*-pQE60-*yjiY* strains were grown in the presence of 50 μM CFSE-labelled dipeptide (images shown are for Lys–Val alone). After 48 h, cells were stained with DAPI and the number of CFSE-labelled dipeptide-containing cells was evaluated by CLSM. The left panels show merged DAPI (blue) and CFSE (green) channels. The right panels show the CFSE channel alone. Bar, 20 μm. (b) Quantification of images. Percentage of CFSE-labelled cells out of the total number of cells was calculated for the dipeptides Lys–Val and Ser–Lys. The data shown are a compilation of a minimum of two biological replicates. Statistical analysis was performed using one-way ANOVA (see Methods).

important adhesin known in *Salmonella* (Dibb-Fuller *et al.*, 1999; Haiko & Westerlund-Wikström, 2013), the reduced transcription of flagellar assembly genes could be the reason behind defective adhesion of the Δ*yjiY* strain.

yjiY-dependent adhesion is mediated by flagella

Flagella-mediated adhesion is established not only in *Salmonella*, but also in many other pathogens (Bucior *et al.*, 2012; Girón *et al.*, 2002; Haiko & Westerlund-Wikström, 2013; Inglis *et al.*, 2003; Tasteyre *et al.*, 2001). The downregulation of flagellar gene expression observed in the Δ*yjiY* strain led to the speculation that *yjiY*-dependent adhesion could be mediated by flagella. Δ*yjiY* was in fact found to be immotile in the motility assays on swim and swarm agar (Fig. 8a). Imaging by TEM (Fig. 8b) and CLSM (Fig. S7) revealed that Δ*yjiY* indeed lacked flagella, unlike the WT strain. Adhesion (Fig. 5 and 6) and invasion assays (Fig. S4) performed with the Δ*fliC* strain confirmed that aflagellated *Salmonella* actually adhered poorly to the host cells irrespective of the cell type and had reduced per cent invasion compared with the WT strain (Fig. S4). This also agrees with the reported role of flagella in the invasion of host cells by *Salmonella* (Haiko & Westerlund-Wikström, 2013). The exogenous expression of *fliC*, by introducing the construct pTrc-*fliC* in Δ*yjiY*, helped the mutant adhere better to RAW 264.7 (Fig. 5) and HeLa cells (Fig. 6). Hence, *yjiY* is required for complete flagellar assembly for ultimately facilitating adhesion of *Salmonella* to host cells.

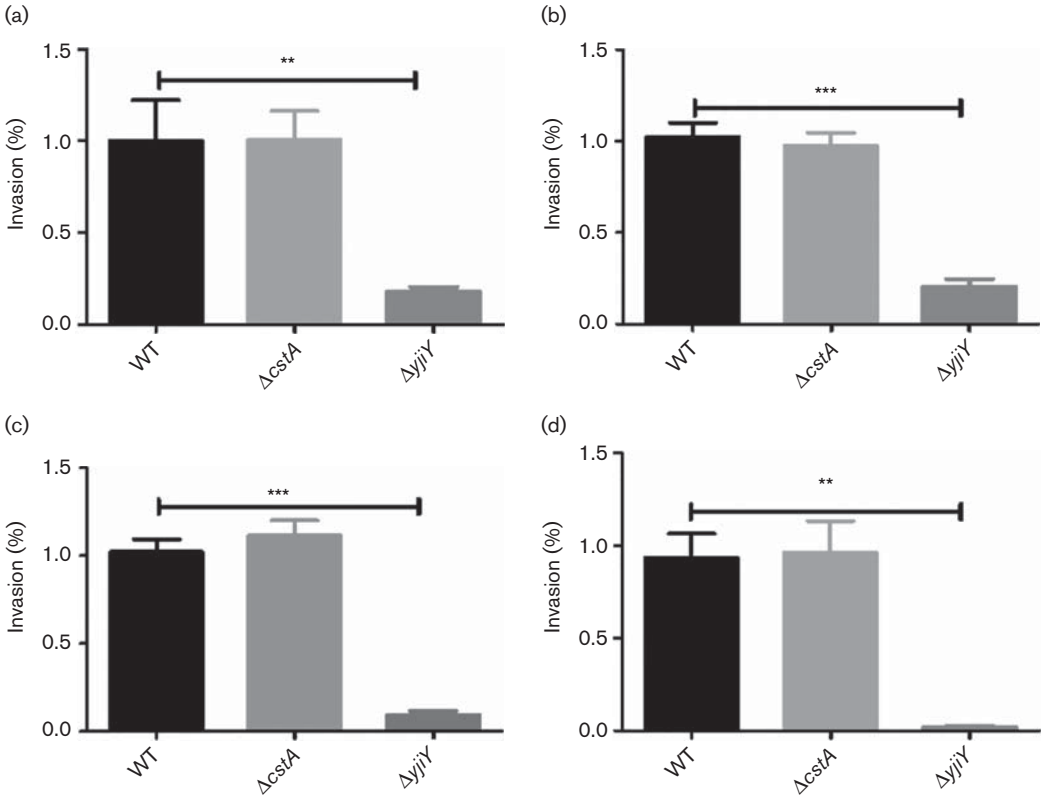

Fig. 4. Invasion of various cell lines. (a) RAW 264.7, (b) polarized Caco-2, (c) Intestine-407 and (d) HeLa cells were infected with the WT, Δ*cstA* and Δ*yjiY* strains. At 1 h post-infection, cells were lysed and plated on LB agar. Per cent invasion with respect to the inoculum used for infection was calculated from the c.f.u. values. The data represent a compilation of three biological replicates. Statistical analysis was performed using one-way ANOVA (see Methods).

Expression of class III flagellar genes is downstream to *yjiY*

The flagellar regulon comprises three temporal classes of genes: class I, II and III. Early (class I) genes act as master regulators for the expression of middle (class II) genes that ultimately control the expression of late (class III) genes which are required for the final steps in the assembly of flagella (Chilcott & Hughes, 2000). Whilst the expression of most of the class III genes was reduced by ∼10-fold in the Δ*yjiY* strain, the class I and class II genes were moderately altered (Table 4). We confirmed these results with qRT-PCR for a few of the class III genes (*fliC*, *fljB* and *cheM*), one class II gene (*fliM*) and one class I gene (*flhD*) (Fig. 9). As the expression of both flagellar subunits, *fliC* as well as *fljB*, was downregulated, the phenomenon seems to be universal for all class III genes and independent of flagellar phase variation (Bonifield & Hughes, 2003). Also, complementation with just the flagellar subunit *fliC* could rescue the adhesion defect of Δ*yjiY* (Figs 5 and 6), implying that the later stages of flagellar assembly mediated by class III genes are downstream to the function of *yjiY*. To understand this phenomenon in further detail, the expression of *fliA* was checked at the RNA level. *fliA* encodes sigma factor 28 that controls the transcription of class III genes (Chilcott & Hughes, 2000). There was no significant change observed in the level of *fliA* RNA in the Δ*yjiY* strain with respect to the WT strain (Fig. 9). This suggests that *yjiY* might be involved in the post-transcriptional regulation of *fliA* to ultimately affect the transcription of class III genes.

In vivo significance of *yjiY*

As flagellum is one of the major virulence factors of *Salmonella* (Haiko & Westerlund-Wikström, 2013; Stecher *et al.*, 2004), the role of *yjiY* in *Salmonella* pathogenesis attracted our attention. The importance of flagella in *Salmonella*-mediated colitis in mice is well established (Stecher *et al.*, 2004). Therefore, we evaluated the ability of the Δ*yjiY* strain to induce colitis in mice using the method of pretreatment with streptomycin (Barthel *et al.*, 2003). The bacterial burden in the caecum of mice showed that Δ*yjiY* colonized the caecum significantly less than the WT strain, which was similar to the phenotype shown by

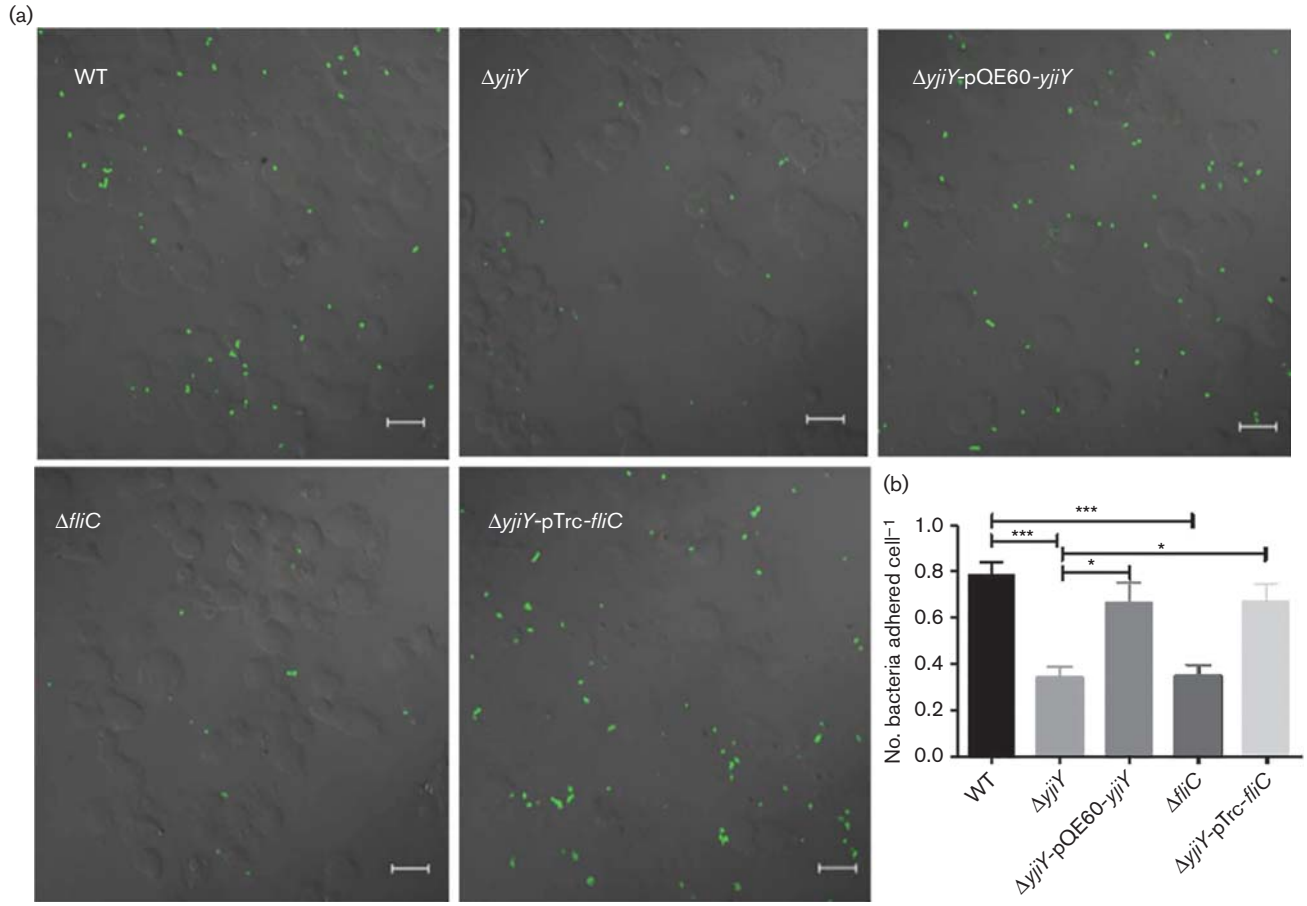

Fig. 5. Adhesion on RAW 264.7 cells. (a) Adhesion assay on monolayers of RAW 264.7 cells was carried out at m.o.i. 50 for the WT, Δ*yjiY*, Δ*yjiY*-pQE60-*yjiY*, Δ*fliC* and Δ*yjiY*-pTrc-*fliC* strains. Adhered bacteria were stained with Alexa Fluor 488 (shown in green). (b) Quantification of images. The total number of adhered bacteria was divided by the total number of host cells to give the number of adhered bacteria per cell. Counting was done for a minimum 10 fields for each sample. The data shown here are a compilation of three biological replicates. Statistical analysis was performed using one-way ANOVA (see Methods).

aflagellated strain Δ*fliC* (Fig. 10a). As flagellar motility is essential for *Salmonella* to travel to the Peyer's patches (Jones *et al.*, 1992), we also compared the bacterial burden of Δ*yjiY* to that of WT in the Peyer's patches of the mouse model. Reduced colonization of Peyer's patches by Δ*yjiY* at early time points of infection confirmed that *yjiY* is required for invading and colonizing the intestine of mouse (Fig. S8). However, systemic infection in the mouse model did not depend on *yjiY*, as shown by bacterial burden in the liver and spleen after 5 days of infection in BALB/c mice (Fig. 10b). This was expected as the presence of flagella is dispensable for systemic infection by *Salmonella* in the mouse model (Schmitt *et al.*, 2001). As reported earlier, we assessed the ability of the *cst* mutants to infect the nematode animal model *C. elegans*. In contrast to what we observed in mammalian system, Δ*yjiY* did not show any significant difference from the WT strain (Fig. S9). However, the Δ*cstA* strain was unable to colonize the intestine of the nematode, unlike WT (Fig. S9), verifying that *cstA* is required by *Salmonella* for infecting *C. elegans* successfully.

DISCUSSION

The link of metabolism-associated genes with the pathogenesis of *Salmonella* forms an exciting field of ongoing research in *Salmonella* biology (Garmory & Titball, 2004; Kim *et al.*, 2013; Steeb *et al.*, 2013). Peptide transporters represent a major part of the metabolic system of bacterial pathogens, including *Salmonella*, as host-derived peptides are required for their survival (Steeb *et al.*, 2013; Vorwerk *et al.*, 2014). The role of peptide transporters in virulence of many bacterial pathogens has been reported previously (Cundell *et al.*, 1995; McNab & Jenkinson, 1998; Moraes *et al.*, 2014; Podbielski & Leonard, 1998; Samen *et al.*, 2004; Slamti & Lereclus, 2002). In *Salmonella*, the significance of peptide transporters in cell motility (Abouhamad

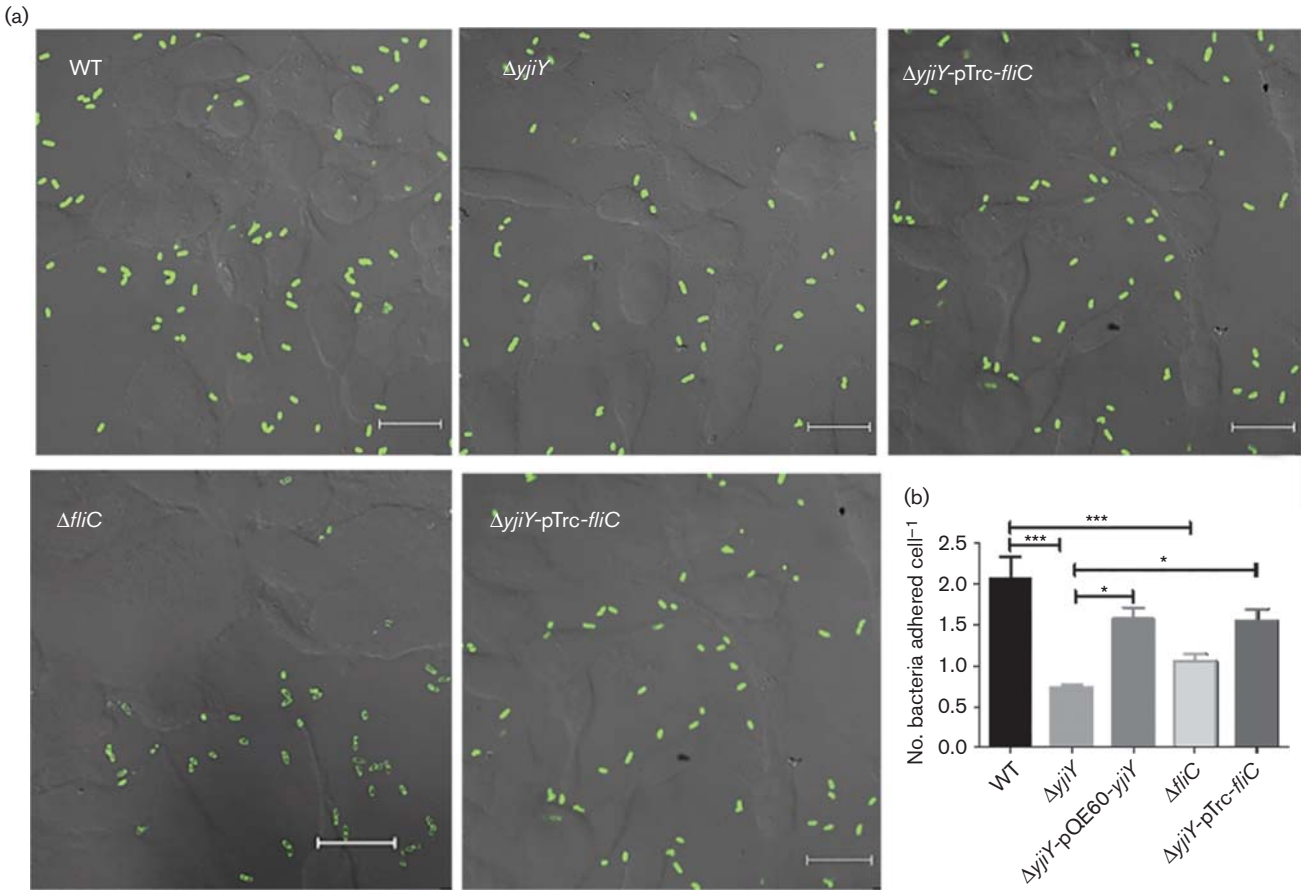

Fig. 6. Adhesion on HeLa cells. (a) Adhesion by CLSM. Late-exponential-phase cultures of the WT, Δ*yjiY*, Δ*yjiY*-pQE60-*yjiY*, Δ*fliC* and Δ*yjiY*-pTrc-*fliC* strains were allowed to infect monolayers of HeLa cells at m.o.i. 50. Adhered bacteria were stained with Alexa Fluor 647 (shown in green). (b) Quantification of images. The total number of bacteria adhered to cells was divided by the total number of host cells to give the number of adhered bacteria per cell. Counting was done for a minimum 10 fields for each sample for three biological replicates. Statistical analysis was performed using one-way ANOVA (see Methods).

et al., 1991) and resistance to antimicrobial peptides (Parra-Lopez *et al.*, 1993) has been described. Clearly, peptide transporters in *Salmonella* are not destined for mere provision of nutrients and are performing alternate crucial cellular functions. Carbon starvation (*cst*) genes, a group of putative peptide transporters, are known to be required for survival of *E. coli* (Dubey *et al.*, 2003; Kraxenberger *et al.*, 2012; Schultz *et al.*, 1988; Schultz & Matin, 1991) as well as *Salmonella* (Kraxenberger *et al.*, 2012; Wong *et al.*, 2013) during the stationary phase, but their role in peptide utilization has not been completely dissected.

We have now verified the role of *cst* genes in peptide utilization for growth in *Salmonella* by using phenotype microarray analysis. Both the *cst* genes are involved in utilizing several peptides with a specific order of amino acids. The difference exhibited in the kind of peptides utilized by both *cst* genes shows their differential role in peptide metabolism. As the array did not contain longer peptides than tripeptides, it is difficult to say if the function of these genes is limited to metabolism of dipeptides only. The difference shown in respiration kinetics in the phenotype microarray analysis was more remarkable in the Δ*yjiY* strain than the Δ*cstA* strain and the level of expression of *yjiY* during the stationary phase was much higher than *cstA* (Fig. S1B). Hence, we conclude that *yjiY* is more important than *cstA* for peptide metabolism. The peptides that allowed better growth of Δ*yjiY* in the phenotype microarray analysis are either taken up by the upregulated alternate peptide transporters in Δ*yjiY* or act as alternate substrates for the YjiY transporter which could be toxic for *Salmonella* and thus supported cellular growth only in the absence of *yjiY*. Although the major role predicted for these genes is transport of peptides, this has never been investigated. The use of fluorescent peptide uptake assay, which is more reliable than the method using radiolabelled peptides as the fluorescent cells can be visualized without any background signal and minute difference in

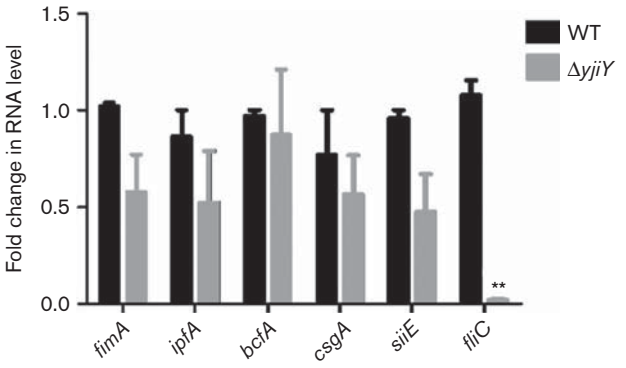

Fig. 7. qRT-PCR of major adhesion-associated genes. RNA was isolated from cultures of the WT and Δ*yjiY* strains grown to OD_{600} 1.5 in LB (late exponential phase), and used for qRT-PCR for the *fimA*, *lpfA*, *bcfA*, *csgA*, *siiE* and *fliC* genes. The level of expression is represented in terms of fold change in RNA level with respect to that in WT. The data are a compilation of three biological replicates. Statistical analysis was performed using one-way ANOVA (see Methods).

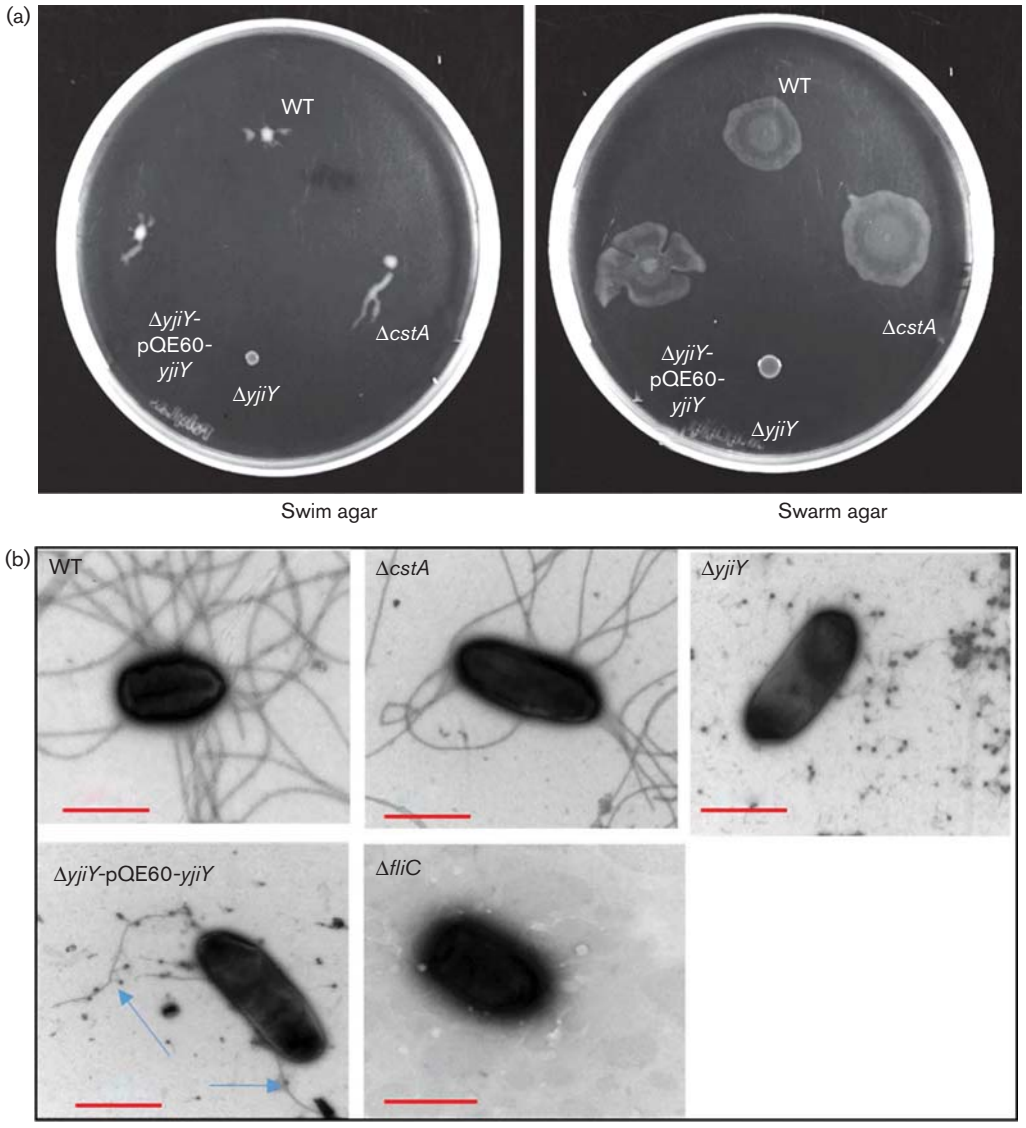

Fig. 8. Flagella formation depends on *yjiY*. (a) Motility on swim and swarm agar. Overnight cultures of the WT, Δ*cstA*, Δ*yjiY* and Δ*yjiY*-pQE60-*yjiY* strains were spotted on swim and swarm agar and allowed to grow for 4–6 h before imaging. (b) Detection of flagella by TEM imaging. Overnight cultures of the WT, Δ*cstA*, Δ*yjiY*, Δ*yjiY*-pQE60-*yjiY* and Δ*fliC* strains, grown in LB, were stained with 1 % uranyl acetate and imaged by TEM. Bar, 1 μm.

Table 4. Expression of genes in the flagellar regulon in the Δ*yjiY* strain

The values given in the microarray data were analysed to obtain the fold change in the expression of the genes listed here with respect to the expression in the WT strain.

Class of flagellar genes	Gene name	Fold change
Class I	*flhC*	0.553879
	flhD	0.631518
	flgA	1.233742
Class II	*flgB*	0.948324
	flgC	0.791714
	flgD	0.680089
	flgE	0.50601
	flgF	0.528996
	flgG	0.451586
	flgH	0.475427
	flgI	0.551147
	flgJ	0.582673
	flhA	1.469843
	flhB	0.961696
	flhE	1.261298
	fliE	1.411088
	fliF	0.765199
	fliG	0.591232
	fliH	0.600215
	fliI	0.608903
	fliJ	0.483599
	fliK	0.359211
	fliL	0.535926
	fliM	0.541806
	fliN	0.580157
	fliO	0.734918
	fliP	0.730023
	fliQ	0.545653
	fliR	0.466273
	fliY	0.521326
	fliZ	0.132655
Class III	*flgK*	0.11997
	flgL	0.125916
	flgM	0.146596
	flgN	0.119729
	fliA	0.211499
	fliB	0.185694
	fliC	0.094856
	fliD	0.091654
	fliS	0.123631
	fliT	0.169222
	fljA	0.643811
	fljB	0.41956
	cheA	0.147245
	cheB	0.108488
	cheM	0.090152
	cheR	0.095337
	cheW	0.124728
	cheY	0.090973
	cheZ	0.120613
	motA	0.190461
	motB	0.168218
	aer	0.067992

the per cent uptake between the WT and the mutant could be evaluated, helped us to establish that *yjiY* contributes to cellular metabolism in *Salmonella* by transporting specific peptide substrates.

In contrast to the role in *Salmonella*-mediated killing of *C. elegans* (Tenor *et al.*, 2004), *cstA* neither participates in adhesion of mammalian cells nor in the colonization of the mammalian gut. However, the homologous gene *yjiY* was shown to be important for colonizing the mouse gut, but not for infecting *C. elegans*. This implies that *cstA* and *yjiY* are required to colonize the gut of two different host systems, demonstrating an example of adaptability of *Salmonella* to various hosts. Whilst the virulence-associated functions dependent on *yjiY* are mediated by flagella, the mechanism behind the effect of *cstA* on the

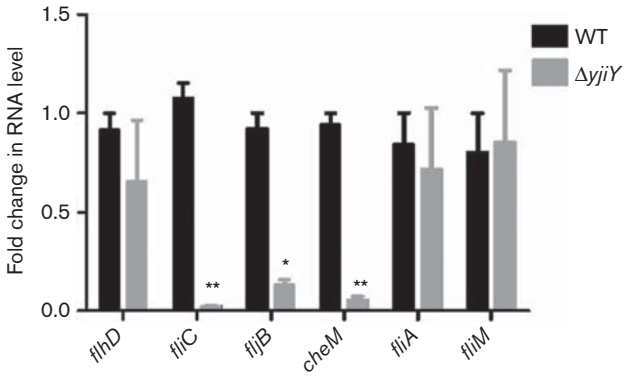

Fig. 9. Expression of genes involved in flagellar biosynthesis. Bacterial cultures grown to OD_{600} 1.5 in LB were used for RNA isolation. qRT-PCR was done for the *flhD*, *fliC*, *fljB*, *cheM*, *fliA* and *fliM* genes. The level of expression is represented in terms of fold change in RNA level with respect to that in the WT strain. Statistical analysis was performed using one-way ANOVA (see Methods) for a minimum of two biological replicates for each sample.

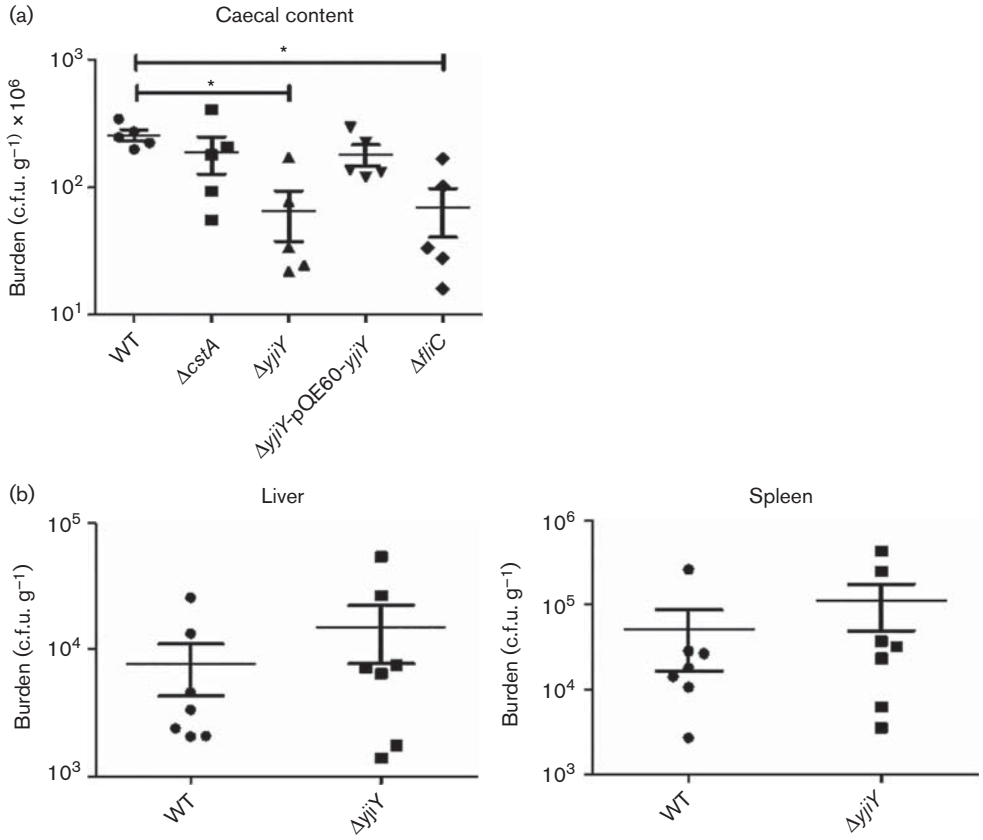

Fig. 10. *In vivo* significance of *yjiY*. (a) Colitis in the mouse model. BALB/c mice (6–8 weeks old) were infected orally with 10^8 c.f.u. of the WT, Δ*cstA*, Δ*yjiY*, Δ*yjiY*-pQE60-*yjiY* and Δ*fliC* strains (n=5 for each group). Bacterial burden in the caecal content was evaluated after sacrificing the mice at 24 h post-infection. One-way ANOVA and Newman–Keuls multiple comparison post-test were used to calculate statistical significance (see Methods). (b) Systemic infection in the mouse model. BALB/c mice (6–8 weeks old) were infected orally with 10^7 c.f.u. of the WT and Δ*yjiY* strains (n=7 for each strain). At 5 days post-infection, mice were sacrificed and bacterial burden was evaluated for the organs indicated. Statistical significance was calculated by Student's *t*-test and the Mann–Whitney post-test (see Methods).

pathogenesis of *Salmonella* remains to be clarified. Our observation of reduced colonization of the intestine of mice and *C. elegans* by Δ*yjiY* and Δ*cstA* strains, respectively, implies that the function of *cst* genes may not be restricted to rescue *Salmonella* from starvation during the stationary phase, and may be extended to mediate a cross-talk between metabolism and pathogenesis of *Salmonella*.

Adhesion of some Gram-positive pathogens to host cells was previously shown to be dependent on the presence of peptide transporters (McNab & Jenkinson, 1998; Moraes *et al.*, 2014), but the mechanism is not completely understood. We have clarified the mechanism behind *yjiY*-dependent adhesion of *Salmonella* to host cells. The expression of the late flagellar genes is reduced in the absence of *yjiY* and the phenotype of defective adhesion can be reversed by expressing the class III gene *fliC* exogenously, indicating that expression of only class III genes is downstream to *yjiY*. The transcription of class III genes depends on the secretion of the anti-sigma factor FlgM outside of the cell after the completion of hook–basal body formation, releasing sigma factor 28 and allowing the transcription of class III genes to take place (Karlinsey *et al.*, 2000). Therefore, it is conceivable that *yjiY* targets the transcription of class III genes after the hook–basal body complex is assembled. As the transcription sigma factor 28 (*fliA*) was found to be unaffected in Δ*yjiY*, the step of post-transcription regulation of FliA by FlgM seems to be targeted by *yjiY*. After mining the microarray data, we found that *mgtC* was upregulated in Δ*yjiY*. MgtC is one of the major virulence factors of *Salmonella* which functions by binding to F-ATPase of *Salmonella* to modulate ATP generation (Lee *et al.*, 2013; Pontes *et al.*, 2015; Thompson *et al.*, 2011). The similarity in the structures of flagellar F-ATPase and target F-ATPase of *mgtC*

(Kishikawa *et al.*, 2013) provides a hint about interference in the process of flagellar assembly by *mgtC* in Δ*yjiY*. The process of assembly of flagellum, including the secretion of FlgM and thereby release of FliA, depends on the functioning of flagellar F-ATPase (Karlinsey *et al.*, 2000). Hence, it is possible that the increased level of *mgtC* in Δ*yjiY* might hinder the functioning of flagellar F-ATPase to block the release of FliA and thereby inhibit the transcription of class III genes.

We conclude that the two carbon starvation (*cst*) genes in *Salmonella* form transport systems for importing specific exogenous peptides which aid in cellular growth. The *yjiY* gene affects flagellar biosynthesis required for adhesion of *Salmonella* to mammalian cells. The role of *yjiY* in cellular adhesion is extended to colonization of mouse intestine *in vivo*. This sort of inter-relationship between pathogenesis and metabolism opens a platform for the study of similar genes, which may in turn unravel the molecular mechanism behind various stages of pathogenesis.

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