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The role of *stj* fimbrial operon in the intestinal persistence of *Salmonella* Typhimurium in mice

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Abstract: *Salmonella* Typhimurium contains 13 operons coding for fimbriae with unique binding specificities to host epithelial surfaces. *stj* operon is only detected in *S.* Typhimurium genome suggesting that Stj fimbria may effect serovar-specific virulence characteristics. In this study, the role of *stj* fimbrial operon in the long-term persistence of *S.* Typhimurium was identified by competitive infection experiment in genetically resistant mouse (CBA) model system. Knock-out mutation of *stjA* (major subunit of the Stj fimbria) gene reduced recovery of *S.* Typhimurium from fecal samples and its colonization to spleen, cecum and mesenteric lymph nodes over a 34-day time period ($p < 0.05$). This data indicate that *stj* fimbrial operon has a role in long-term intestinal persistence of *S.* Typhimurium in CBA mice.

Key words: *S.* Typhimurium; *stj*; fimbria; intestinal persistence.

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; CFU, colony forming unit; LB, Luria-Bertani; MLN, mesenteric lymph nodes; PBS, phosphate buffered saline.

Introduction

The genus *Salmonella* comprises two species, *Salmonella enterica* and *Salmonella bongori*. *S. enterica* includes seven subspecies of clinical importance for humans causing million of cases of food borne disease in the world every year (Linam & Gerber 2007; Grassl & Finlay 2008). *S. enterica* serotype Typhimurium (*S.* Typhimurium) is a motile pathogen that has adapted to invading and surviving for long periods in its human and mouse hosts (Tükel et al. 2005, 2006; Weening et al. 2005). *S.* Typhimurium related human gastroenteritis is initiated by the colonization of intestinal epithelium followed by invasion of M cells and enterocytes (Ledeboer et al. 2006). Fimbriae play a critical role in virulence of *Salmonella* by allowing these bacteria to interact with intestinal epithelium (Edwards et al. 2000; Raffatellu et al. 2006).

S. Typhimurium genome contains 13 fimbrial operons of the chaperone/usher class: *agf* (*csg*), *fim*, *lpf*, *pef*, *bcf*, *stb*, *ste*, *std*, *stf*, *sth*, *sti*, *saf* and *stj* (Weening et al. 2005; Tükel et al. 2007; Chessa et al. 2008b). Expression of these operons is regulated by environmental signals, such as temperature, iron, pH, osmolarity, aliphatic amino acids, oxygen levels and carbon source (Nicholson & Low 2000). Only two type I fimbriae, encoded by the *fim* operon (Stolpe et al. 1994) and thin aggregative fimbriae encoded by *agf* (*csg*) gene cluster (Romling et al. 1998) are highly expressed *in vitro*. The lack of the *in vitro* expression of the other fimbrial operons including *stj* has prevented identification of their functions. *stj* fimbrial operon was identified only by sequence analysis of *S.* Typhimurium genome (Porwollik et al. 2002).

In this study, we present the evidence that Stj fimbria enhances *S.* Typhimurium survival in the mouse model system.

Material and methods

Bacterial strains and media
S. Typhimurium LT2 is a standard laboratory strain which was first isolated by Lilleengen (1948). *S.* Typhimurium IR715 (Stojilkovic et al. 1995) is a spontaneous nalidixic acid-resistant virulent variant of ATCC 14028. Strain AJB715 is a *phoN* mutant derivative of IR715 and has been previously described (Kingsley et al. 2004). *Escherichia coli* strain S17-1λ*pir* has been described elsewhere (Grund & Weber 1988). Bacteria were cultured aerobically at 37 °C in Luria-Bertani (LB) broth. Antibiotics were used at the following concentrations: kanamycin, 100 μg/mL; and nalidixic acid, 50 μg/mL.

DNA manipulations
The *stjA* gene upstream and downstream fragments were amplified to construct *S.* Typhimurium *stj* mutant by using the primers:
5'GCAGATCTGCGGAAGTGATTTCACGG3',

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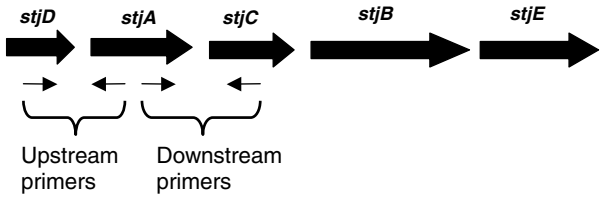

Fig. 1. PCR amplified *stjA* gene upstream and downstream fragments (lanes; 1: 1 kb DNA ladder; 2: *stjA* upstream fragment, 650 bp; and 3: *stjA* downstream fragment, 1041 bp).

5'CGGTCGACCCAAATGACATGTAATGCGCGGGTC GGG3' and
5'CGGTCGACCTCCAGCATTCACATGGAATATTCA ACACC3',
5'GCTCTAGATCCAGTTTCACACCATTGTCGTC3'.

Amplified PCR fragments were cloned into the vector PCR 2.1 (TOPO TA Cloning Kit, Invitrogen). The two fragments flanking the *stj* operon were then cloned into the suicide vector pGP704 (Kinder et al. 1993) digested with *XBa*I and *Bgl*II. A kanamycin resistance gene cassette from plasmid pUC4-KIXX (Barany 1985) was cloned into *Sal*I restriction site where the two *stj*A fragments connected on the plasmid pGP704. The resulting plasmid was introduced into *E. coli* S17-1*λpir* and then conjugated into IR715. The inserts of the resulting plasmid were sequenced by RefGen (METU Teknokent, Ankara). Conjugants were selected on LB agar plates containing kanamycin (100 μg/mL) and nalidixic acid (50 μg/mL). The donor strain *E. coli* S17-1*λpir* cannot grow on this medium because of lack of diaminopimelate. Southern blot analysis was performed by using an internal *stj*A fragment as a DNA probe to confirm the insertion of pGP704 into *S.* Typhimurium IR715 chromosome by homologous recombination. This recombinant was termed as MA44.

Competitive infection experiments

Four resistant CBA mice were inoculated orally with 2×10^8 colony forming unit (CFU) of a 1:1 mixture of AJB715 and MA44 in a volume of 0.1 mL phosphate buffered saline (PBS). Fecal and organ samples – cecum, ileum and mesenteric lymph nodes (MLN) – were homogenized and serially diluted for determination of CFU and input ratio. Double plating was performed on LB agar plates containing kanamycin (100 μg/mL), nalidixic acid (50 μg/mL) and 30 μg/mL of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) in order to distinguish phoN$^-$ (alkaline phosphatase) AJB715 and phoN$^+$ MA44. Data obtained from competitive infection experiments were normalized by dividing the output ratio (CFU mutant/CFU wild-type) by the input ratio (CFU mutant/CFU wild-type). All data were converted logarithmically prior to the calculation of geometric means and statistical analysis. Statistical significance was determined by using Student's t test. P values of < 0.05 were considered as statistically significant.

Results and discussion

To determine if *stj* operon conferred persistence to *S.* Typhimurium in genetically resistant mice (CBA) we constructed a *stj* mutant, termed as MA44, by chromosomal insertion of suicide plasmid pGP704. To this end, upstream and downstream DNA regions of the main subunit gene (*stj*A) of *stj* operon were amplified (Fig. 1) and cloned into pGP704. A kanamycin resistance gene cassette from plasmid pUC4 was inserted between upstream and downstream fragments of *stj*A gene on pGP704 in *Sal*I restriction site and resulting plasmid was transferred from *E. coli* strain S17-1*λpir* (Simon et al. 1983) into *S.* Typhimurium IR715 strain by conjugation. *stj* operon knockout mutants contained kanamycin gene cassette insertion via homologous recombination of *stj*A gene fragments in which common on the suicide plasmid pGP704 and *S.* Typhimurium IR715 chromosome were selected as conjugants which were dap$^+$ and kanamycin resistant. Insertion of the suicide plasmid containing kanamycin gene cassette between *stj*A gene fragments was confirmed by Southern blotting (Fig. 2) by using genomic DNA of *S.* Typhimurium and DNA specific probes for major subunit gene (*stj*A) of *stj* operon.

Fig. 2. Southern blot of genomic DNA isolated from *S.* Typhimurium strains IR715 (wild type, lane 1) and MA44 (*stjA* mutant, lane 2), detected with DNA probe specific for *stjA* gene.

To investigate the role of *stj* fimbrial operon in intestinal persistence, competitive inhibition experiments were done with a group of four CBA mice. An equal mixture of the *stj* mutant (MA44) and the strain *S.* Typhimurium AJB715 ($\sim10^6$ CFU/mL) was used to inoculate the CBA mice group intragastrically. *S.* Typhimurium strain AJB715 carries a mutation in phoN gene, a gene encoding nonspecific acid phosphatase and controls the blue color phenotype of cells on agar plates containing the chromogenic substrate BCIP (Kier et al. 1977). Inactivation of *pho*N gene has been shown not to effect intestinal colonization and virulence of *S.* Typhimurium for mice (Kingsley et al. 2004). Growth on LB agar plates supplemented with BCIP thus provided an easy means to distinguish the *stj* mutants.

Mice fecal pellets were collected at 3, 7, 9, 14, 17, 21, 25, 28, 30 and 34 days after infection. Numbers of competing strain AJB715 (phoN$^-$, white colonies) and the *stj* fimbrial mutant MA44 (phoN$^+$, blue colonies) were determined by spreading serial ten-fold dilutions of fecal homogenates on LB agar plates supplemented with BCIP and appropriate antibiotics. Over 34-day time period of experiment, *stj* mutant MA44 was recovered at significantly lower numbers ($p<0.05$) than

Table 1. Competitive infection results in mice fecal samples.[a]

Days	AJB715 (CFU/mL)	MA44 (CFU/mL)	AJB715/MA44 (CFU/mL)	Normalized to input ratio	Log
Day 3					
mouse 1	1	610	0.001639	0.0020708	−2.68387219
mouse 2	8000	145000	0.055172	0.0696915	−1.15682037
mouse 3	6800	900	7.555556	9.5438596	0.97972404
mouse 4	1	20	0.05	0.0631579	−1.19957235
Day 7					
mouse 1	10	590	0.016949	0.0214095	−1.66939437
mouse 2	25200000	2.6e-07	0.965517	1.2196007	0.08621767
mouse 3	46000	23000	2	2.5263158	0.40248764
mouse 4	1	1	1	1.2631579	0.10145764
Day 9					
mouse 1	200	500	0.4	0.5052632	−0.29648237
mouse 2	9800000	8200000	1.195122	1.5096277	0.17886986
mouse 3	8000	145000	0.055172	0.0696915	−1.15682037
mouse 4	1	1	1	1.2631579	0.10145764
Day 14					
mouse 1	1	110000	9.09e-06	1.148e-05	−4.93993504
mouse 2	2370000	1590000	1.490566	1.8828203	0.27480886
mouse 3	360000	2450000	0.146939	0.1856069	−0.73140594
mouse 4	10	500	0.02	0.0252632	−1.59751236
Day 17					
mouse 1	550000	80000	6.875	8.6842105	0.93873034
mouse 2	4300000	1600000	2.6875	3.3947368	0.53080611
mouse 3	1500000	600000	2.5	3.1578947	0.49939765
mouse 4	40000	315000	0.126984	0.160401	−0.79479292
Day 21					
mouse 1	290000	1330000	0.218045	0.2754254	−0.559996
mouse 2	26700000	9100000	2.934066	3.7061885	0.56892751
mouse 3	37000	56000	0.660714	0.8345865	−0.07852866
mouse 4	380000	530000	0.716981	0.9056604	−0.04303463
Day 25					
mouse 1	1130000	50000	22.6	22.6	1.45556608
mouse 2	19900000	4900000	4.061224	4.061224	0.71011464
mouse 3	10	20	0.5	0.5	−0.19957235
mouse 4	328000	63000	5.206349	5.206349	0.81799094
Day 28					
mouse 1	10000	2000	5	6.3157895	0.80042765
mouse 2	18800000	4700000	4	5.0526316	0.70351763
mouse 3	2010000	208000	9.663462	12.206478	1.08659036
mouse 4	5400000	200000	27	34.105263	1.5328214
Day 30					
mouse 1	1900	1200	1.583333	2	0.30103
mouse 2	42000000	9600000	4.375	5.5263158	0.7424357
mouse 3	4000	700	5.714286	7.2180451	0.85841959
mouse 4	1900	100	19	24	1.38021124
Day 34					
mouse 1	70	10	7	8.8421053	0.94655568
mouse 2	26100000	2600000	10.03846	12.680162	1.1031248
mouse 3	40	1	40	50.526316	1.70351763
mouse 4	1	1	40	50.526316	1.70351763

Statistical analysis

Day	Mean	Standard deviation	Standard error	*P* value
3	−1.01513522	1.50756	0.753781	0.1133548
7	−0.26980785	0.94435	0.472177	0.2942346
9	−0.29324381	0.61222	0.306112	0.1875372
14	−1.74851112	2.261	1.130498	0.0864517
17	0.2935353	0.75264	0.376321	0.2325156
21	−0.02815795	0.46265	0.231323	0.0435964
25	0.69602482	0.68168	0.340841	0.0435964
28	1.03083926	0.37208	0.186038	0.0007291
30	0.82052413	0.44372	0.221859	0.0050538
34	1.36417894	0.39701	0.198507	0.000234

[a] Data given as CFU/mL, multiply by 5 to get CFU/organ.

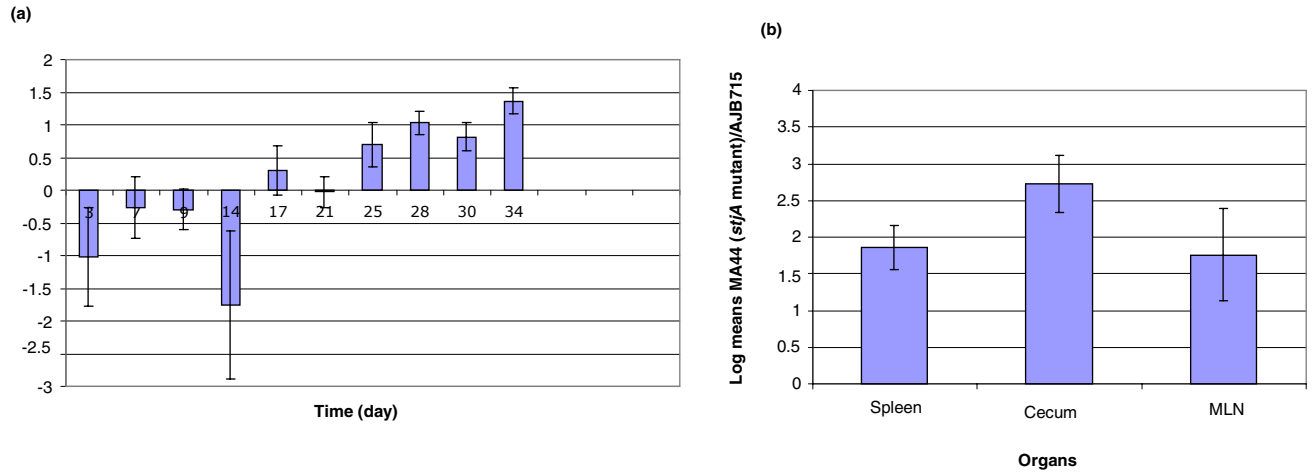

Fig. 3. Competitive infection experiment results from CBA mice feces (a) and organs (b). Mice were infected with a mixture of equal amounts of AJB715 and *stj* mutant (MA44). Ratios of MA44/AJB715 recovered from feces over a 34-day time period and spleen, cecum and mesenteric lymph nodes (MLN) of mice at 34 days after infection were plotted as log means.

Table 2. Competitive infection results in mice organ samples.[a]

Organs	AJB715 (CFU/mL)	MA44 (CFU/mL)	AJB715/MA44 (CFU/mL)	Normalized to input ratio	Log
Spleen					
mouse 1	520	1	520	656.8421053	2.81746098
mouse 2	45	1	45	56.84210526	1.75467015
mouse 3	110	5	22	27.78947368	1.44388032
mouse 4	20	1	20	25.26315789	1.40248764
Cecum					
mouse 1	108	1	108	136.4210526	2.1348814
mouse 2	3500	4	875	1105.263158	3.04346569
mouse 3	7800	120	65	82.10526316	1.914371
mouse 4	5000	1	5000	6315.789474	3.80042765
MLN					
mouse 1	1	1	1	1.263157895	0.10145764
mouse 2	890	1	890	1124.210526	3.05084765
mouse 3	115	12	9.583333333	12.10526316	1.08297424
mouse 4	500	1	500	631.5789474	2.80042765

Statistical analysis

Organs	Mean	Standard deviation	Standard error	P value
Spleen	1.85462477	0.66085386	0.296347024	0.000682347
Cecum	2.72328643	0.86858364	0.38949939	0.000382134
MLN	1.75892679	1.40924322	0.631947633	0.023381781

[a] Data given as CFU/mL, multiply by 5 to get CFU/organ.

the competing strain AJB715 (Fig. 3a, Table 1). At the entire peripheral site samples (spleen, cecum and MLN), the mutant was less able to colonize than the wild-type strain AJB715 ($p < 0.05$) (Fig. 3b, Table 2). These results indicate that *stj* fimbrial operon has a role in the colonization and long-term intestinal persistence of *S.* Typhimurium in mice.

Bacteria are capable to expressing a variety of surface structures, such as fimbriae in response to diverse environmental conditions. Fimbriae are thin, proteinaceous, polymeric non-motile structures which mediate adhesion of the cell to host tissue or to bacteria of same or different species. These interactions are committed steps leading to subsequent colonization of epithelial surfaces, entry into host cells or bacterial conjugation (Duguid et al. 1966; Cogan et al. 2004; Ledeboer et al. 2006; Chessa et al. 2008a). Many of gene clusters corresponding to fimbrial system are present in genomes of enteric bacteria. Genetic analyses have shown that a series of horizontal transfer and deletion events have resulted in a high distribution pattern of fimbrial operons observed for the *Salmonella* serovars (Baumler et al. 1997; Humphries et al. 2001; Cogan et al. 2004; Forest et al. 2007). The *in vivo* expression of many fimbrial structures, such as Bcf, Fim, Lpf, Pef, Stb, Stc, Std, Stf and Sti has been demonstrated from *S.* Typhimurium infected bovine ileal loops; most of them were not, however, expressed *in vitro* (Romling et al. 1998; Humphries et al. 2003, 2005; Chessa et al. 2008b).

Stj fimbrial operon was identified by DNA sequence

analysis of *S.* Typhimurium LT2 genome (McClelland et al. 2001; Porwollik et al. 2002). There is not any information about the role of Stj fimbria in virulence or persistence of *S.* Typhimurium in the host system. This is the first report showing that the *stj* fimbrial operon affects colonization and long-term persistence of *S.* Typhimurium in mouse model systems. Additional experiments, such as cloning and *in vitro* expression of Stj fimbria in homologous and heterologous hosts and determination of regulation characteristics of *stj* operon in *S.* Typhimurium are needed to strengthen this prediction.

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References

Barany F. 1985. Single-stranded hexameric linkers: a system for in-phase insertion mutagenesis and protein engineering. Gene **37:** 111–123.

Baumler A.J., Tsolis R.M. & Heffron F. 1997. Fimbrial adhesins of *S.* Typhimurium. Adv. Exp. Med. Biol. **412:** 149–158.

Chessa D., Dorsey C.W., Winter M.G. & Baumler A.J. 2008a. Binding specificity of *Salmonella* plasmid-encoded fimbriae assessed by glycomics. J. Biol. Chem. **28:** 8118–8124.

Chessa D., Winter M.G., Nuccio S.P., Tükel C. & Baumler A.J. 2008b. RossE repress *std* fimbrial expression in *Salmonella enterica* serotype Typhimurium. Mol. Microbiol. **68:** 573–587.

Cogan T. A., Jørgensen F., Lappin-Scott H. M., Benson C. E., Woodward M. J. & Humphrey T. J. 2004. Flagella and curli fimbriae are important for the growth of *Salmonella enterica* serovars in hen eggs. Microbiology **150:** 1063–1071.

Duguid J.P., Anderson E.S. & Campbell I. 1966. Fimbriae and adhesive properties in *Salmonellae*. J. Pathol. Bacteriol. **92:** 107–137.

Edwards R.A., Schifferli D.M. & Maloy S.R. 2000. A role for *Salmonella* fimbriae in intraperitoneal infections. Proc. Natl. Acad. Sci. USA **97:** 1258–1262.

Forest C., Faucher S.P., Poirier K., Houle S., Dozois C.M. & Daigle F. 2007. Contribution of the stg fimbrial operon of *Salmonella enterica* Serovar Typhi during interaction with human cells. Infect. Immun. 75: 5264–5271.

Grassl G.A. & Finlay R.B. 2008. Pathogenesis of enteric *Salmonella* infections. Curr. Opin. Gastroenterol. **24:** 22–26.

Grund S. & Weber A. 1988. A new type of fimbriae on *Salmonella typhimurium*. Zentralbl. Veterinarmed. **B35:** 779–782.

Humphries A.D., DeRidder S. & Baumler A.J. 2005. *Salmonella enterica* serotype Typhimurium fimbrial proteins serve as antigens during infection of mice. Infect. Immun. **73:** 5329–5338.

Humphries A.D., Raffatellu M., Winter S., Weening E.H., Kingsley R.A., Droleskey R., Zhang S., Figueiredo J., Khare S., Nunes J., Adams L.G., Tsolis R.M. & Baumler A.J. 2003. The use of flow cytometry to detect expression of subunits encoded by 11 *Salmonella enterica* serotype Typhimurium fimbrial operons. Mol. Microbiol. **48:** 1357–1376.

Humphries A.D.,Townsend S.M., Kingsley R.A., Nicholson T.L., Tsolis R.M. & Baumler A.J. 2001. Role of fimbriae as antigens and intestinal colonization factors of *Salmonella* serovars. FEMS Microbiol. Lett. **201:** 121–126.

Kier L.D., Weppelman R.M. & Ames B.N. 1977. Regulation of two phosphatases and a cyclic phosphodiesterase of *Salmonella typhimurium*. J. Bacteriol. **130:** 420–428.

Kinder S.A., Badger J.L., Bryant G.O., Pepe J.C. & Miller V.L. 1993. Cloning of the *Yen*I restriction endonuclease and methyltransferase from *Yersinia enterocolitica* serotype O8 and construction of a transformable R-M + mutant. Gene **136:** 271–275.

Kingsley R.A., Abi Ghanem D., Puebla-Osorio N., Keestra A.M., Berghman L. & Baumler A.J. 2004. Fibronectin binding to the *Salmonella enterica* Serotype Typhimurium ShdA autotransporter protein is inhibited by a monoclonal antibody recognizing the A3 repeat. J. Bacteriol. **186:** 4931–4939.

Ledeboer N.A., Frye J.G., McClelland M. & Jones B.D. 2006. *Salmonella enterica* serovar Typhimurium requires the Lpf, Pef, and Tafi fimbriae for biofilm formation on HEp-2 tissue culture cells andchicken intestinal epithelium. Infect. Immun. **74:** 3156–3169.

Lilleengen K. 1948. Typing of *Salmonella typhimurium* by means of bacteriophage. Acta. Pathol. Microbiol. Scand. **77 (Suppl.):** 2–125.

Linam W.M. & Gerber M.A. 2007. Changing epidemiology and prevention of *Salmonella* infections. Pediatr. Infect. Dis. J. **26:** 747–748.

McClelland M., Sanderson K.E., Spieth J., Clifton S.W., Latreille P., Courtney L., Porwollik S., Ali J., Dante M., Du F., Hou S., Layman D., Leonard S., Nguyen C., Scott K., Holmes A., Grewal N., Mulvaney E., Ryan E., Sun H., Florea L., Miller W., Stoneking T., Nhan M., Waterston R. & Wilson R.K. 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:** 852–856.

Nicholson B. & Low D. 2000. DNA methylation-dependent regulation of Pef expression in *Salmonella typhimurium*. Mol. Microbiol. **35:** 728–742.

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

Raffatellu M., Chessa D., Wilson R.P., Tükel Ç., Akçelik M. & Baumler A.J. 2006. Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. Infect. Immun. **74:** 19–27.

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

Simon R., Priefer U. & Puhler A. 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. Bio/Technology **1:** 784–791.

Stojilkovic I., Baumler A.J. & Heffron F. 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the cchA cchB eutE eutJ eutG eutH gene cluster. J. Bacteriol. **177:** 1357–1366.

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

Tükel Ç., Akçelik M., de Jong M.F., Şimşek Ö., Tsolis R.M. & Baumler A.J. 2007. MarT activates expression of the MisL autotransporter protein of *Salmonella enterica* Serotype Typhimurium. J. Bacteriol. **189:** 3922–3926.

Tükel Ç., Raffatellu M., Chessa D., Wilson R.P., Akçelik M. & Baumler A.J. 2006. Neutrophil influx during non-typhoidal salmonellosis: who is in the driver's seat? FEMS Immunol. Med. Microbiol. **46:** 320–329.

Tükel Ç., Raffatellu M., Humphries A.D., Wilson R.P., Andrews-Polymenis H.L., Gull T., Figueiredo J.F., Wong M.H., Michelsen K.S., Akçelik M., Adams L.G. & Baumler A.J. 2005. CsgA is a pathogen-associated molecular pattern of *Salmonella enterica* serotype Typhimurium that is recognized by Toll-like receptor 2. Mol. Microbiol. **58:** 289–304.

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

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