Title
Loss of very-long O-antigen chains optimizes capsule-mediated immune evasion by Salmonella enterica serovar Typhi.

Permalink
https://escholarship.org/uc/item/25q7f3nr

Journal
mBio, 4(4)

ISSN
2150-7511

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Publication Date
2013-07-16

DOI
10.1128/mbio.00232-13

Peer reviewed
ABSTRACT Expression of capsular polysaccharides is a variable trait often associated with more-virulent forms of a bacterial species. For example, typhoid fever is caused by the capsulated Salmonella enterica serovar Typhi, while nontyphoidal Salmonella serovars associated with gastroenteritis are noncapsulated. Here we show that optimization of the immune evasion properties conferred by the virulence-associated (Vi) capsular polysaccharide involved an additional alteration to the cell envelope of S. Typhi, namely inactivation of the fepE gene, encoding the regulator of very-long-O-antigen chains. Introduction of the capsule-encoding viaB locus into the nontyphoidal S. enterica serovar Typhimurium reduced complement deposition in vitro and intestinal inflammation in a mouse colitis model. However, both phenotypes were markedly enhanced when the viaB locus was introduced into an S. Typhimurium fepE mutant, which lacks very-long-O-antigen chains. Collectively, these data suggest that during the evolution of the S. Typhi lineage, loss of very-long-O-antigen chains by pseudogene formation was an adaptation to maximize the anti-inflammatory properties of the Vi capsule polysaccharide.

IMPORTANCE Genomic comparison illustrates that acquisition of virulence factors by horizontal gene transfer is an important contributor to the evolution of enteric pathogens. Acquisition of complex virulence traits commonly involves horizontal transfer of a large gene cluster, and integration of the gene cluster into the host genome results in the formation of a pathogenicity island. Acquisition of the virulence-associated (Vi) capsule polysaccharide encoded by SPI7 (Salmonella pathogenicity island 7) was accomplished in the human-adapted Salmonella enterica serovar Typhi by inactivation of the fepE gene, encoding the regulator of very-long-O-antigen chains. We show that the resulting loss of very-long-O-antigen chains was an important mechanism for maximizing immune evasion mediated by the Vi capsule polysaccharide. These data suggest that successful incorporation of a capsular polysaccharide requires changes in the cell envelope of the hosting pathogen.

Typhoid fever is a severe systemic infection that presents with fever (1) after an average incubation period of 2 weeks (2). In contrast, gastroenteritis caused by Salmonella enterica serovar Typhimurium is a localized diarrheal disease with an average incubation period of <24 h (3). The swift onset of diarrhea, abdominal pain, and fever during gastroenteritis is explained by the rapid induction of an acute inflammatory response in the intestine, which requires deployment of two type III secretion systems (T3SSs) encoded by Salmonella pathogenicity island 1 (SPI1) and SPI2 (4–6). Although SPI1 and SPI2 are present in S. enterica serovar Typhi (7), the development of intestinal inflammation is slowed markedly during typhoid fever by expression of the virulence-associated (Vi) capsule polysaccharide (8–10). The anti-inflammatory properties of the Vi capsule polysaccharide (11–13) have been proposed to contribute to the long incubation period characteristic of typhoid fever (14, 15).

The biosynthesis of the Vi capsule polysaccharide is encoded by the viaB locus, a 14-kb DNA region located on SPI7, a pathogenicity island present in S. enterica serovar Typhi but absent from Salmonella serovars associated with gastroenteritis (16, 17). The viaB locus contains genes for the regulation (tviA), biosynthesis (tviBCDE), and surface assembly (vexABCDE) of the Vi capsule polysaccharide (18, 19). Expression of the positive regulator TviA is repressed in the intestinal lumen but induced by the two-component system EnvZ/OmpR at an osmolarity encountered in tissue (20, 21). As a result, expression of the Vi capsule polysaccharide is induced when bacteria transit through the intestinal epithelium (22). This regulatory mechanism ensures that S. Typhi is encapsulated by the time it encounters complement. Expression of the Vi capsule polysaccharide inhibits complement deposition (23, 24), because its homopolymeric chains contain approximately 300 residues of \((\alpha-1,4)-2\text{-acetamido}-3\text{-O-acetyl}-2\text{-deoxy}-\alpha-\text{d-galacturonic acid}\) (25), a sugar that does not contain free hydroxyl groups available for ester formation with complement component 3 fragment b (C3b).

Since S. Typhi is strictly human adapted, it is difficult to study the function of its virulence factors in animal models (26). One approach to study the role the Vi capsule polysaccharide plays in vivo has been the introduction of the viaB locus into S. Typhimurium, a natural pathogen of mice (8, 9, 12, 13, 24). However, it is
not known whether horizontal transfer of the viaB locus is sufficient to optimally deploy the encoded capsular polysaccharide for immune evasion. Here we show that for the Vi capsular polysaccharide to confer maximal evasion of complement fixation, it is necessary to change the cell envelope of *S*. *Typhimurium*, a process that also accompanied the evolution of the *S*. *Typhi* lineage.

RESULTS

Very-long O-antigen chains interfere with the function of the Vi capsular polysaccharide in vitro. We performed an *in vitro* assay to determine whether expression of the Vi capsular polysaccharide in *S. enterica* serovars Typhi and Typhimurium had identical effects on inhibiting complement fixation. Consistent with previous reports (24), analysis of bacterial cells by flow cytometry showed that incubation of a noncapsulated *S*. *Typhi* mutant (*viaB* mutant) in 10% human serum resulted in efficient deposition of C3b on the bacterial surface, while complement deposition was markedly reduced in the capsulated *S*. *Typhi* wild-type strain (Ty2) (Fig. 1A and B). Similarly, a capsulated *S*. *Typhimurium* strain in which the *viaB* locus of *S*. *Typhi* was inserted chromosomally into the *phoN* gene (*phoN::viaB* mutant) deposited less complement on its surface than the noncapsulated *S*. *Typhimurium* wild-type strain (IR715) (Fig. 1B and C).

To investigate possible reasons for differences in the efficacy by which the Vi capsular polysaccharide reduced complement deposition in *S*. *Typhi* and *S*. *Typhimurium*, we compared expression of lipopolysaccharide (LPS), a surface structure containing O-antigen repeat units that are known to fix complement (27). LPS molecules contain a lipid A anchor and an oligosaccharide core but differ in the number of O-antigen repeat units that extend from the bacterial surface. In *S*. *Typhimurium*, O-antigen repeat units are composed of a trisaccharide backbone, consisting of /H9251-D-mannose-(1,4)-/H9251-L-rhamnose-(1,3)-/H9251-D-galactose, and a branching sugar (abequose) that is /H9251-(1,3)-linked to D-mannose in the backbone. Consistent with previous reports, *S*. *Typhimurium* exhibited a trimodal distribution in LPS length, including short-LPS species containing between 1 and 15 O-antigen repeat units, long-LPS species carrying between 16 and 35 O-antigen repeat units, and very-long-LPS species with more than 100 O-antigen repeat units (Fig. 1D) (28–30). O-antigen repeat units of *S*. *Typhi* carry tyvelose as the branching sugar that is /H9251-(1,3)-linked to the backbone but are otherwise identical to those of *S*. *Typhimurium*. Interestingly, in contrast to *S*. *Typhimurium*, *S*. *Typhi* expressed only short-LPS species and long-LPS species, while very-long-LPS species were absent (Fig. 1D).

The *fepE* open reading frame encodes the length regulator of very-long O-antigen chains (28) but is interrupted in the *S*. *Typhi* genome by a stop codon (7). To test whether this mutation in *fepE* is responsible for the lack of LPS species with very-long O-antigen

![FIG 1](https://example.com/figure1.png)

(A to C) Fixation of C3 after incubation of the indicated *S*. *Typhi* and *S*. *Typhimurium* strains (wild type and mutants) in 10% human serum was detected by flow cytometry using an anti-human C3 (α-human C3) FITC conjugate. The experiments shown in panels A and C were repeated 3 times independently with similar outcomes, and a representative example is shown. The average maximum fluorescence intensity (MFI) values ± standard errors (error bars) determined for these three independent experiments are shown in panel B. (D) Silver-stained SDS-PAGE of LPS preparations from the indicated *S*. *Typhimurium* and *S*. *Typhi* strains. The positions of short, long, and very-long O-antigen chains are indicated to the left of the gel. A magnification of the region showing long and very-long O-antigen chains is shown on the right, and the presence of very-long O-antigen chains in *S*. *Typhimurium* strains is indicated by black arrows.
chains in S. Typhi, we introduced a plasmid carrying the cloned S. Typhimurium fepE gene (pRC37). In contrast to the wild-type S. Typhi, an S. Typhi strain carrying a plasmid encoding the S. Typhimurium fepE gene (pRC37) produced LPS species with very-long O-antigen chains (Fig. 2A). These data suggested that conversion of fepE into a pseudogene was responsible for the inability of S. Typhi to produce very-long O-antigen chains, which confirmed previous results (31).

We reasoned that very-long O-antigen chains containing an estimated 100 copies of a repeat unit composed of a trisaccharide backbone (28) might rival in length the homopolymeric chains of the Vi capsular polysaccharide comprising approximately 300 sugar residues (25). Therefore, we wanted to test whether the reduced efficacy by which the Vi capsular polysaccharide diminished complement deposition in S. Typhimurium was due to the presence of very-long O-antigen chains, which confirmed previous results (31).

Lack of very-long O-antigen chains enhances capsule-mediated suppression of colitis. We used the mouse colitis model (5) to investigate the biological relevance of our observations. In this model, mice are preconditioned by treatment with streptomycin, which disrupts the resident microbiota. Subsequent inoculation with S. Typhimurium results in acute cecal inflammation, which is an animal model for human gastroenteritis (reviewed in reference 26). Groups of streptomycin-pretreated mice were either mock infected or inoculated with the wild-type S. Typhimurium or a fepE mutant, phoN::viaB mutant, or fepE phoN::viaB mutant, and the cecum and colon contents were collected 72 h after infection. While the wild-type S. Typhimurium and phoN::viaB mutant were recovered in similar numbers from cecal contents, a small but significant (P < 0.05) reduction in bacterial numbers was observed for strains lacking very-long O-antigen chains (i.e., the fepE mutant and fepE phoN::viaB mutant) (Fig. 3A). These data were consistent with our previous observa-
tion that very-long O-antigen chains are required for optimal survival of *S*. Typhimurium in the lumen of the inflamed gut (32).

To assess how the presence of very-long O-antigen chains influences inflammatory responses in the cecal mucosa, we determined mRNA levels of inflammatory markers, including gamma interferon (IFN-γ) encoded by the *Ifng* gene, tumor necrosis factor alpha (TNF-α) encoded by the *Tnfa* gene, interleukin-22 (IL-22) encoded by the *Il22* gene, keratinocyte-derived cytokine (KC) encoded by the *Kc* gene, and macrophage-inducible protein 2 (MIP-2) encoded by the *Mip2* gene, by quantitative real-time PCR. Although the wild-type *S*. Typhimurium was recovered in significantly greater numbers from colon contents than the *fepE* mutant (Fig. 3A), both strains elicited similar levels of *Ifng*, *Tnfa*, *Il22*, *Kc*, and *Mip2* expression in the cecal mucosa (Fig. 3B to F). Thus, the presence of very-long O-antigen chains did not alter expression levels of inflammatory markers elicited by *S*. Typhimurium in the mouse colitis model. Introduction of the *S*. Typhi *viaB* locus into the *S*. Typhimurium genome (*phoN::viaB* mutant) significantly (*P < 0.05*) reduced mRNA levels of *Ifng*, *Tnfa*, *Il22*, *Kc*, and *Mip2* compared to those elicited by infection with the *phoN::viaB* mutant (Fig. 3B to F). Thus, in the absence of very-long O-antigen chains, the *viaB* locus suppressed expression of inflammatory markers to a significantly (*P < 0.05*) greater extent than in their presence. Reduced expression of inflammatory markers was independent of the bacterial burden, because the *fepE* mutant and the *fepE phoN::viaB* mutant were recovered in similar numbers (Fig. 3A), whereas the former elicited significantly (*P < 0.05*) higher mRNA levels of *Ifng*, *Tnfa*, *Il22*, *Kc*, and *Mip2* than the latter (Fig. 3B to F).

We next performed a blinded analysis of histopathological changes observed in the cecal mucosa 72 h after infection to determine the biological consequences of expressing very-long O-antigen chains and/or the Vi capsular polysaccharide. Ceca from mice infected with the wild-type *S*. Typhimurium or a *fepE* mutant were devoid of any contents and had severe gross pathological changes, characterized by reduced size with thickening of the cecal wall. Histopathological evaluation revealed epithelial erosion, neutrophil infiltration in the mucosa, and edema in the submucosa. In contrast, ceca from mock-infected mice did not show gross pathological changes or overt histopathology (Fig. 4). Introduction of the *S*. Typhi *viaB* locus into the *S*. Typhimurium genome (*phoN::viaB* mutant) resulted in a small but significant (*P < 0.05*) reduction in the severity of histopathological changes compared to mice infected with the wild-type *S*. Typhimurium or a *fepE* mutant. Remarkably, infection with a *fepE phoN::viaB* mu-
tart resulted in a significant ($P < 0.05$) reduction in the severity of histopathological changes compared to the $phoN:\viaB$ mutant. Overall, the results from this histopathological analysis (Fig. 4) substantiated results from expression analysis of inflammatory markers in the cecal mucosa (Fig. 3B to F) and supported the concept that optimal suppression of intestinal inflammation by the $\viaB$ locus requires an absence of very-long O-antigen chains.

**DISCUSSION**

The fact that the vast majority of the 2,587 known serovars of *S. enterica* are zoonotic and associated with a localized, self-limiting gastroenteritis in immune-competent individuals (33) suggests that human-restricted specialists associated with systemic febrile illnesses, such as *S. enterica* serovar Typhi or Paratyphi A, evolved from ancestral zoonotic organisms that caused gastroenteritis (34). While the *S. enterica* species is estimated to be 40 to 63 million years old (35), *S. Typhi* represents a clonal lineage that emerged only recently, between 10,000 and 150,000 years ago (36, 37). After the *S. Typhi* lineage had passed through approximately 75% of its evolutionary history, it exchanged some 23% of its genome by horizontal gene transfer with the *S. Paratyphi* A lineage, presumably during coexistence in a shared human reservoir (38). Subsequent to this large-scale genetic exchange, which might mark the origin of typhoid and paratyphoid fever, the lineages of *S. Typhi* and *S. Paratyphi* A became isolated again, and both of their genomes subsequently accumulated pseudogenes at an accelerated rate (39).

Many pseudogenes present in *S. Typhi* encode functions required for the gastrointestinal lifestyle of *S. Typhimurium*. For example, the genome of *S. Typhi* strain CT18 contains pseudogenes in 7 of its 11 chaperone/usher-type fimbrial operons (40), which encode adhesins required by *S. Typhimurium* to colonize the intestinal lumen (41). Furthermore, the *S. Typhi* genome carries pseudogenes in operons functioning in anaerobic respiration ($trrS, dmsA, dmsB, narV$, and $narW$) (7, 42), and these functions are required by *S. Typhimurium* to outgrow obligate anaerobic bacteria in the lumen of an acutely inflamed gut during gastroenteritis (43, 44). While these genes are likely maintained in *S. Typhimurium* because they aid in intestinal growth and transmission during gastroenteritis (45), they can be seen as dispensable for the extraintestinal lifestyle of *S. Typhi*, a pathogen that spreads by means of water, milk, and food products contaminated by individuals with chronic gallbladder carriage (46). Thus, pseudogene formation in *S. Typhi* is commonly viewed as a process leading to random losses of genetic functions that are inherited from an ancestral organism associated with gastroenteritis but that are not required for causing typhoid fever in humans.

Our results suggest that, surprisingly, the formation of one pseudogene, namely, a $fepE$ allele interrupted by a stop codon, resulted in a gain of function during the evolution of the *S. Typhi* lineage. Specifically, we show that inactivation of $fepE$ resulted in an enhanced functionality of the Vi capsular polysaccharide. The Vi capsular polysaccharide is an important virulence factor of *S. Typhi* (reviewed in reference 47) and has been developed into a vaccine against typhoid fever (48). Expression of the Vi capsular polysaccharide inhibits complement deposition (23, 24), a process that was more efficient in strains lacking a functional $fepE$ gene. One consequence of complement activation is the formation of a membrane attack complex on the bacterial surface that leads to lysis unless serum resistance mechanisms are deployed. One study reports that the Vi capsular antigen is not required for serum resistance of *S. Typhi* (31), but in this study, bacteria were grown in Luria-Bertani (LB) broth, a condition that represses expression of the $fepE$ gene.

A second consequence of complement deposition and activation of the alternative pathway is the production of anaphylatoxins (C3a and C5a). Anaphylatoxins are potent enhancers of cytokine responses elicited by stimulating the Toll-like receptor 4 (TLR4)/MD2/CD14 receptor complex with LPS (reviewed in reference 50). Thus, suppression of complement activation by the Vi capsular polysaccharide might explain why expression of this virulence factor diminishes the induction of TLR4/MD2/CD14-dependent proinflammatory responses (10–13). Expression of the Vi capsular polysaccharide in *S. Typhimurium* attenuates intestinal inflammation elicited in bovine ligated ileal loops (8) and in

**FIG 4** Streptomycin-pretreated mice were either mock infected or infected with the indicated *S. Typhimurium* strains, and the cecum was collected 72 h after infection. (A) Combined histopathology score of pathological changes observed in sections from the cecum. Each symbol represents the combined histopathology score for an individual animal. The average for each group of mice is indicated by a short line. (B) Representative images of histopathological changes.
the mouse colitis model (9). Interestingly, suppression of intestinal inflammation by the Vi capsular polysaccharide was significantly enhanced when production of very-long O-antigen chains was abrogated by inactivation of the fepE gene. These data suggest that conversion of fepE into a pseudogene enhanced the ability of S. Typhi to suppress or delay intestinal inflammation using the Vi capsular polysaccharide.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** Bacterial strains used in this study are presented in Table 1. A plasmid carrying the S. enterica serovar Typhimurium fepE gene cloned into the low-copy-number vector pWSK29 has been described previously (32). Bacterial cultures were routinely incubated with aeration at 37°C in Luria-Bertani (LB) broth (10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter) or on LB plates (15 g agar per liter). The following antibiotics were added as necessary at appropriate concentrations: chloramphenicol (Cm), 0.03 mg/ml; carbenicillin, 0.1 mg/ml; kanamycin (Kan), 0.05 mg/ml; and nalidixic acid, 0.05 mg/ml.

**Construction of mutants.** The fepE::pGP704 mutation was transduced from S. Typhimurium RC31 into the S. Typhimurium pheN::viaB mutant TH170 using transduction with phage P22 HT int-105 to yield strain RC60.

**Animal experiments.** All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Davis, and performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

**RNA extraction and quantitative real-time PCR.** Cecal tissue was homogenized in a mininadebeater (BioSpec Products), and RNA was extracted by the TRI reagent method (Molecular Research Center) as described previously (53). Reverse transcription was performed using TaqMan reagent (Applied Biosystems), and 2 μl of converted cDNA was used with a 250 nM concentration of primers listed in Table 2 and SYBR green (Applied Biosystems) for real-time PCR in the ViiA 7 system (Life Technologies). Data were analyzed using the comparative threshold cycle method. Transcription levels of Ifng, Tnfa, Il22, Kc, and Mip2 genes were normalized to Gapdh mRNA (encoding glyceraldehyde-3-phosphate dehydrogenase [GAPDH]).

**Histopathology.** Cecal tissues were formalin fixed, sectioned, stained with hematoxylin and eosin (H&E) and submitted to a veterinary pathologist for blinded scoring using a scale described previously (52). Representative images of tissue sections were taken using an Olympus BX41 microscope.

**Analysis of Vi and LPS expression and C3 deposition by flow cytometry.** Detection of Vi and LPS expression by flow cytometry was performed as described previously (9) using the DNA-specific stain propidium iodide, rabbit anti-Vi serum, or anti-0:4 (1:250 dilution; Becton Dickinson) and goat anti-rabbit fluorescein isothiocyanate (FITC) conjugate (1:250 dilution; Jackson ImmunoResearch). Binding of complement component 3 (C3) was determined by flow cytometry as described previously (24) using the DNA-specific stain propidium iodide, human serum (10% dilution; Quidel), and fluorescein isothiocyanate (FITC)-conjugated goat anti-human C3b monoclonal antibody (1:250 dilution; MP Biomedicals). All samples were analyzed on an LSRII instrument (BD).

**Statistical analysis.** Relative abundance values of S. Typhimurium strains and fold changes in mRNA levels were converted logarithmically (log10) prior to statistical analysis using a one-tailed parametric test (Student’s t test). A P value of <0.05 was considered to be significant.

**ACKNOWLEDGMENT**

We acknowledge support by Public Health Service grant AI044170 to A.J.B.

**REFERENCES**

1. Nasrallah SM, Nassar VH. 1978. Enteric fever: a clinicopathologic study of 104 cases. Am. J. Gastroenterol. 69:63—69.
2. Olsen SJ, Bleasdale SC, Magnano AR, Landrigan C, Holland BH, Tauxe RV, Mintz ED, Luby S. 2003. Outbreaks of typhoid fever in the United States, 1960—99. Epidemiol. Infect. 130:13–21.
3. Grunwald GM, Palmer SR. 1992. Incubation period, severity of disease, and infecting dose: evidence from a Salmonella outbreak. Am. J. Epidemiol. 136:1369—1377.
4. Tsolis RM, Adams LG, Ficht TA, Bäumler AJ. 1999. Contribution of Salmonella typhimurium virulence factors to diarrheal disease in calves. Infect. Immun. 67:4879—4885.
5. Barthel M, Hapfelmeier S, Quintanilla-Martínez L, Kremer M, Rohde M, Hogardt M, Pfeffer K, Rüssmann H, Hardt WD. 1997. Pretreatment of mice with streptomycin provides a Salmonella enterica serovar Typhi- murium colitis model that allows analysis of both pathogen and host. Infect. Immun. 71:2839—2858.
6. Coburn B, Li Y, Owen D, Vallance BA, Finlay BB. 2005. Salmonella enterica serovar Typhimurium pathogenicity island 2 is necessary for complete virulence in a mouse model of infectious enterocolitis. Infect. Immun. 73:3219—3227.
7. Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall K, Bentley SD, Holden MT, Sebaihia M, Baker...
10. Jansen AM, Hall LJ, Clare S, Goulding D, Holt KE, Grant AJ, Mastroeni P, Dougan G, Kingsley RA. 2011. The Vi capsule prevents Toll-like receptor 4 recognition of Salmonella. Cell. Microbiol. 13:527–535.

11. Hirose K, Ezaki T, Miyake M, Li T, Khan AQ, Kawamura Y, Yokoyama T. 2010. A Salmonella typhimurium-Typhi Vi capsular antigen is expressed after the bacterium enters the ileal mucosa. Infect. Immun. 78:3367–3374.

12. Polymenis HL, Bäumler AJ. 2009. The capsule-encoding viaB locus encodes FepE as a second Wzz. Mol. Microbiol. 73:4342–4350.

13. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Keestra AM, Winter SE, Xavier MN, Tsolis RM, Tolstikov V, Bäumler AJ. 2012. Very long O-antigen chains enhance fitness during Salmonella-induced colitis by increasing bile resistance. PLoS Pathog. 8(5):1002918. doi:10.1371/journal.ppat.1002918.

14. Skelton J, Stevens K, Whitehead S, Barrell BG. 1982. Studies on the bimodal pattern of relatedness between the Salmonella Paratyphi A and Typhi genomes: convergence or divergence by homologous recombination? Genome Res. 175:393–404.

15. Winter SE, Thiennimitr P, Gomez G, Khare S, Lawhon SD, Raffatellu M, Bäumler AJ. 2008. Very long O-antigen chains enhance fitness during Salmonella-induced colitis by increasing bile resistance. PLoS Pathog. 4(7):e1002131. doi:10.1371/journal.ppat.1002131.

16. Beltrán RA, Alifano P, Bififi E, Hull SI, Hull RA. 1992. Nucleotide sequences of the genes regulating O-polyosaccharide antigen chain length (rol) from Escherichia coli and Salmonella typhimurium: protein homology and functional complementation. J. Bacteriol. 174:5228–5236.

17. Winter SE, Haraguchi GE, Hull RA, Hull SI. 1991. Regulation by a novel protein of the bimodal distribution of lipopolysaccharide in the outer membrane of Escherichia coli. J. Bacteriol. 173:5699–5704.

18. Beltrán RA, Silva C, Carter JA, Hoare A, Alvarez SA, Blondel CJ, Zaldívar M, Valvano MA, Contreras I. 2008. Growth-phase regulation of lipopolysaccharide O-antigen chain length influences serum resistance in serovars of Salmonella. J. Med. Microbiol. 57:938–946.

19. Bravo D, Silva C, Carter JA, Hoare A, Alvarez SA, Blondel CJ, Zaldívar M, Valvano MA, Contreras I. 2008. Growth-phase regulation of lipopolysaccharide O-antigen chain length influences serum resistance in serovars of Salmonella. J. Med. Microbiol. 57:938–946.

20. Deng W, Liou SR, Plunkett G, III, Mayhew GF, Rose DJ, Burland V, Masse GL, White-Kauffmann-Le Minor scheme. Res. Microbiol. 161:1–29.

21. Psuvide P, Mogensen P, Mikkelsen F, Bäumler AJ. 2002. Salmonella typhi, the causative agent of typhoid fever, is approximately 50,000 years old. Infect. Genet. Evol. 2:33–45.

22. Roumagnac P, Weil FX, Doelecke B, Baker S, Brisse S, Chinh NT, Le Dinh T, Achtman M, Parkhill J. 2006. Molecular phylogeny of the salmonellae: relationships among Salmonella species and subspecies determined from four housekeeping genes and evidence of lateral gene transfer events. J. Bacteriol. 190:7060–7067.

23. Psuvide P, Mogensen P, Mikkelsen F, Bäumler AJ. 2002. Salmonella typhi, the causative agent of typhoid fever, is approximately 50,000 years old. Infect. Genet. Evol. 2:33–45.

24. Roumagnac P, Weil FX, Doelecke B, Baker S, Brisse S, Chinh NT, Le Dinh T, Achtman M, Parkhill J. 2006. Molecular phylogeny of the salmonellae: relationships among Salmonella species and subspecies determined from four housekeeping genes and evidence of lateral gene transfer events. J. Bacteriol. 190:7060–7067.

25. Psuvide P, Mogensen P, Mikkelsen F, Bäumler AJ. 2002. Salmonella typhi, the causative agent of typhoid fever, is approximately 50,000 years old. Infect. Genet. Evol. 2:33–45.

26. Psuvide P, Mogensen P, Mikkelsen F, Bäumler AJ. 2002. Salmonella typhi, the causative agent of typhoid fever, is approximately 50,000 years old. Infect. Genet. Evol. 2:33–45.
controlled by virulence factors and indigenous intestinal microbiota. Infect. Immun. 76:403–416.

46. Stone WJ. 1912. The medical aspect of chronic typhoid infection (typhoid bacillus carriers). Am. J. Med. Sci. 143:544–557.

47. Wangdi T, Winter SE, Bäumler AJ. 2012. Typhoid fever: "you can’t hit what you can’t see." Gut Microbes 3:88–92.

48. Robbins JD, Robbins JB. 1984. Reexamination of the protective role of the capsular polysaccharide (Vi antigen) of Salmonella typhi. J. Infect. Dis. 150:436–449.

49. Hashimoto Y, Li N, Yokoyama H, Ezaki T. 1993. Complete nucleotide sequence and molecular characterization of ViaB region encoding Vi antigen in Salmonella typhi. J. Bacteriol. 175:4456–4465.

50. Haas PJ, van Strijp J. 2007. Anaphylatoxins: their role in bacterial infection and inflammation. Immunol. Res. 37:161–175.

51. Winter SE, Winter MG, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tisolis RM, Stewart VJ, Bäumler AJ. 2013. Host-derived nitrate boosts growth of E. coli in the inflamed gut. Science 339:708–711.

52. Thiennimitr P, Winter SE, Winter MG, Xavier MN, Tolstikov V, Huseby DL, Sterzenbach T, Tisolis RM, Roth JR, Bäumler AJ. 2011. Intestinal inflammation allows Salmonella to utilize ethanolamine to compete with the microbiota. Proc. Natl. Acad. Sci. U. S. A. 108:17480–17485.

53. Stojiljkovic I, Bäumler AJ, Heffron F. 1995. Ethanolamine utilization in Salmonella typhimurium: nucleotide sequence, protein expression, and mutational analysis of the cchA cchB eucE eucJ eucG eucH gene cluster. J. Bacteriol. 177:1357–1366.