

# Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota

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Conventional wisdom holds that microbes support their growth in vertebrate hosts by exploiting a large variety of nutrients. We show here that use of a specific nutrient (ethanolamine) confers a marked growth advantage on *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) in the lumen of the inflamed intestine. In the anaerobic environment of the gut, ethanolamine supports little or no growth by fermentation. However, *S. Typhimurium* is able to use this carbon source by inducing the gut to produce a respiratory electron acceptor (tetrathionate), which supports anaerobic growth on ethanolamine. The gut normally converts ambient hydrogen sulfide to thiosulfate, which it then oxidizes further to tetrathionate during inflammation. Evidence is provided that *S. Typhimurium*'s growth advantage in an inflamed gut is because of its ability to respire ethanolamine, which is released from host tissue, but is not utilizable by competing bacteria. By inducing intestinal inflammation, *S. Typhimurium* sidesteps nutritional competition and gains the ability to use an abundant simple substrate, ethanolamine, which is provided by the host.

diarrhea | gastroenteritis | metabolism

The pathogen *Salmonella enterica* serotype Typhimurium (*S. Typhimurium*) is a common cause of gastroenteritis (1), a diarrheal illness characterized by acute intestinal inflammation and the presence of neutrophils in stool samples (2). The pathogen triggers inflammation in the gut by using its virulence factors, two type III secretion systems (T3SS), termed T3SS-1 and T3SS-2, to invade the intestinal epithelium and survive in tissue phagocytes, respectively (3–6). Inflammation supports growth of *S. Typhimurium* in the intestinal lumen (7–9), thereby promoting transmission of the pathogen to the next susceptible host (10). However, it remains unknown what nutrients promote growth of *S. Typhimurium* in the inflamed intestine.

Because of extensive metabolic redundancies and access to a surprisingly diverse range of nutrients in vivo, many metabolic pathways in *S. Typhimurium* are not essential for growth in tissue (11). Although the array of nutrients is certainly also diverse in the gut lumen, it seems likely that competing microbes limit the usefulness of many of the substrates that can support fermentative growth in this anaerobic environment. Furthermore, inflammatory diarrhea changes this environment dramatically by removing potential nutrients from the gut lumen and reducing the diversity of available nutrients (12). Nutrient sources in the inflamed intestinal lumen include the mucin and enterocytes released from the tips of villi. Phosphatidylethanolamine is the most abundant phospholipid in membranes of released enterocytes (13). Ethanolamine, a nutrient derived from phosphatidylethanolamine, is present in the intestine of healthy calves (14). Here we investigated whether ethanolamine utilization supports growth of *S. Typhimurium* in an inflamed intestinal lumen.

## Results

**Ethanolamine Utilization Is Not Required for Virulence in the Mouse Typhoid Model.** The *S. Typhimurium* *eut* gene cluster encodes 17 genes required for the degradation and utilization of ethanolamine (*eutS*, *-P*, *-Q*, *-T*, *-D*, *-M*, *-N*, *-E*, *-J*, *-G*, *-H*, *-A*, *-B*, *-C*, *-L*, *-K*, *-R*) (15, 16) (Fig. 1A). To investigate the role of ethanolamine utilization in *S. Typhimurium*, we constructed an *eutC* mutant (PT100) in *S. Typhimurium* strain IR715. This mutant lacks ethanolamine ammonia lyase, which catalyzes the first step in ethanolamine utilization. Because ethanolamine has been shown to be present in the mammalian intestine (14), we investigated whether this nutrient supports growth of *S. Typhimurium* in the murine gut lumen. Mice infected with *S. Typhimurium* develop a typhoid fever-like disease, but acute neutrophilic inflammation does not develop in the intestine (mouse typhoid model) (Fig. S1A). In this mouse typhoid model, the *eutC* mutant (PT100) showed full virulence. Equal numbers of *S. Typhimurium* wild-type (IR715) and the *eutC* mutant (PT100) were recovered from intestinal contents and organs of mice (C57BL/6) 4 d after inoculation with an equal mixture of both strains (Fig. S1B). This result was consistent with a previous report showing that inactivation of the *eut* operon does not markedly attenuate *S. Typhimurium* virulence in the mouse typhoid model (16). Thus, data derived from infection of inbred susceptible mice support the idea that ethanolamine utilization is not essential for systemic infection.

**Tetrathionate Respiration Supports Anaerobic Growth on Ethanolamine in Vitro.** We next investigated the ability of *S. Typhimurium* to grow on ethanolamine anaerobically in vitro. In the absence of an exogenous electron acceptor, ethanolamine only poorly supported anaerobic growth by fermentation (Fig. 1B and C). However, in the presence of the respiratory electron acceptor tetrathionate ( $S_4O_6^{2-}$ ), the *S. Typhimurium* wild-type (IR715), but not the *eutC* mutant (PT100), was able to anaerobically grow on ethanolamine as the sole carbon source (Fig. 1B and C). Ethanolamine did not support anaerobic growth of *S. Typhimurium* in the absence of tetrathionate (Fig. 1B and C). These data were consistent with previous studies demonstrating that under anaerobic conditions *S. Typhimurium* can use ethanolamine as a carbon source only in the presence of tetrathionate as a respiratory electron acceptor (17). Tetrathionate respiration does not provide a growth benefit for *S. Typhimurium* in the mouse typhoid model (8, 18). One limitation

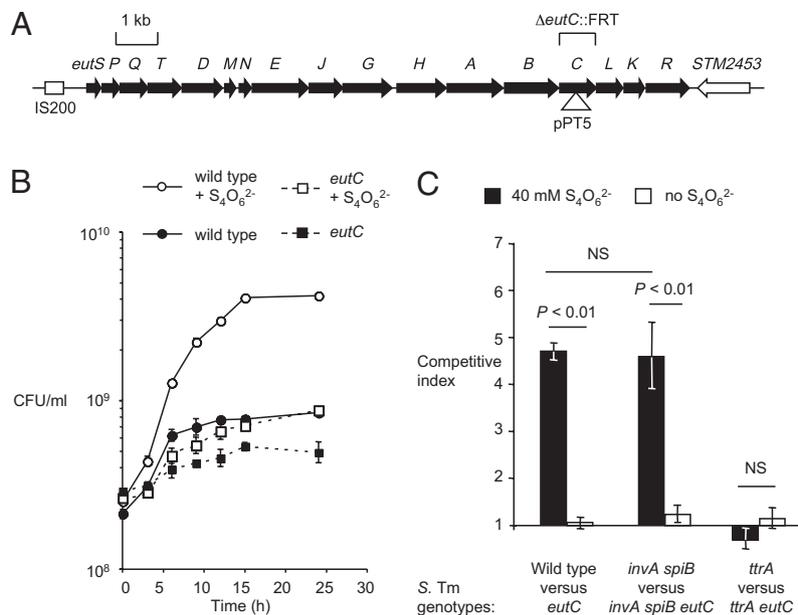
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**Fig. 1.** Anaerobic growth of *S. Typhimurium* on ethanolamine as the sole carbon source in vitro. (A) Schematic drawing of the *eut* locus in the chromosome of the *S. Typhimurium* strain LT2. The locations, orientations and sizes of genes are indicated by arrows. Mutation in strains PT100 (*eutC*:pPT5) and TT26355 ( $\Delta$ *eutC*::FRT) are indicated. (B) Anaerobic growth curve of the *S. Typhimurium* wild-type (IR715; circles) and an *eutC* mutant (PT100; squares) with ethanolamine as the sole carbon source in the presence (open symbols) or absence (filled symbols) of the respiratory electron acceptor tetrathionate ( $S_4O_6^{2-}$ ). The number of viable bacteria was determined at the indicated time points. (C) Competitive growth of *S. Typhimurium* strains [wild-type (IR715) vs. *eutC* mutant (PT100); *invA spiB* mutant (SPN452) vs. *invA spiB eutC* mutant (PT102); *ttrA* mutant (SW661) vs. *ttrA eutC* mutant (PT106)]. Bacterial numbers were determined 24 h after anaerobic growth with ethanolamine as the sole carbon source in the presence (black bars) or absence (white bars) of tetrathionate. Data represent the geometric mean of three independent experiments  $\pm$  SE. Statistical significance (*P* value) is indicated above the bars.

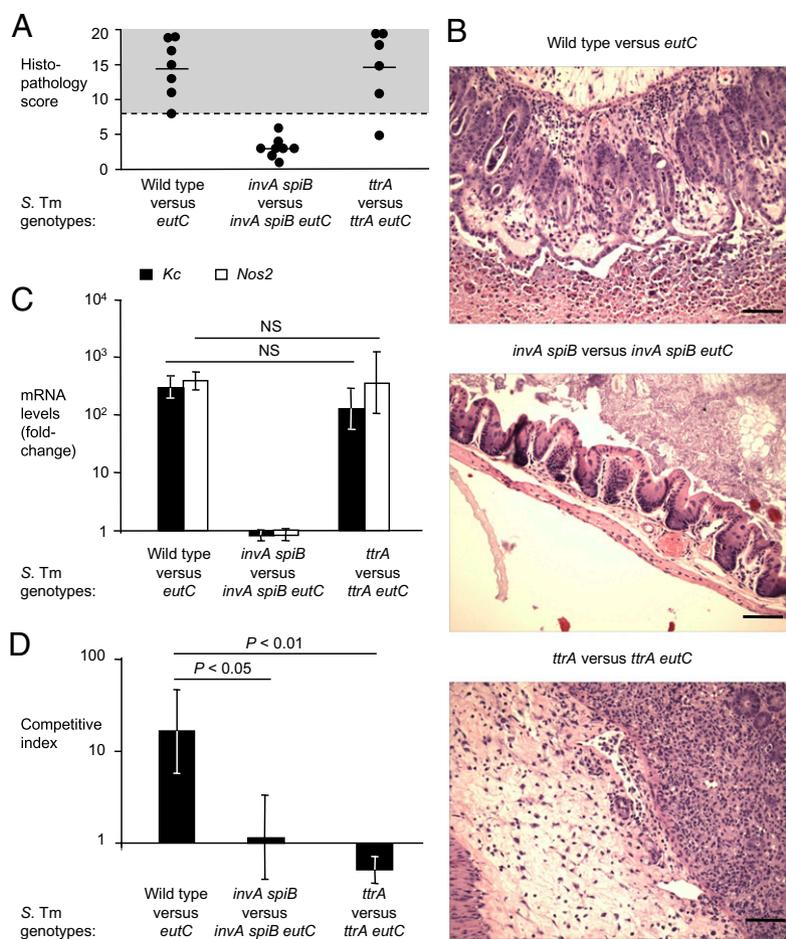
of the mouse typhoid model is that mice show no signs of gastroenteritis. This limitation can be overcome if, before *S. Typhimurium* infection, mice are treated with streptomycin, which results in intestinal inflammation with marked neutrophil transmigration into the lumen (Fig. S1C) (mouse colitis model) (5). In the mouse colitis model, it has recently been shown that neutrophils transminating into the intestinal lumen during inflammation increase the relative luminal abundance of *S. Typhimurium* (7) by oxidizing endogenous sulfur compounds (thiosulfate) to generate tetrathionate (8). These observations suggested that the importance of ethanolamine utilization in vivo should be reevaluated.

***eut* Gene Cluster Confers a Growth Advantage in the Mouse Colitis Model.** Because *S. Typhimurium* requires tetrathionate respiration for ethanolamine utilization under anaerobic conditions in vitro (Fig. 1 B and C) (17), we reasoned that this might also be true under conditions of the mouse colitis model. Streptomycin-pretreated mice (C57BL/6) were orally infected with an equal mixture of the *S. Typhimurium* wild-type strain (IR715) and the *eutC* mutant (PT100). These mice developed acute cecal inflammation (Fig. 2 A and B and Fig. S2B) and showed increased levels of mRNA from the *Kc* gene, encoding a neutrophil chemoattractant, and the *Nos2* gene, encoding inducible nitric oxide synthase (Fig. 2C). A marked enrichment ( $P < 0.05$ ) for the *S. Typhimurium* wild-type strain over the *eutC* mutant was observed 4 d after infection in the colon contents (Fig. 2D and Fig. S1D). A similar phenotype was observed with a *S. Typhimurium* mutant (TT26355) carrying an in-frame deletion of the *eutC* gene ( $\Delta$ *eutC*::FRT), which was not polar on expression of the downstream *eutLKR* genes (Figs. S3 and S4). To determine whether a growth benefit is observed at later time points, genetically resistant mice (129/svJ) were pretreated with streptomycin and infected with an equal mixture of the *S. Typhimurium* wild-type strain (IR715) and the *eutC* mutant (PT100). A marked enrichment ( $P < 0.05$ ) for the *S. Typhimurium* wild-type strain

over the *eutC* mutant was observed 11, 12, and 15 d after infection in the fecal contents (Fig. S5). Growth of the *eutC* mutant (PT100) on ethanolamine in vitro and in the mouse colitis model (C57BL/6) could be restored by either bringing back the wild type *eutC* allele (PT238) or by complementing the strain with an F plasmid carrying the *eut* operon (F' 606) (Fig. S3 A and B). Collectively, these data suggested that ethanolamine utilization provided a luminal growth advantage in the mouse colitis model.

**Ethanolamine Utilization Supports Growth in Inflamed Intestine.** *S. Typhimurium*'s ability to invade and survive in host tissues depends on T3SSs (T3SS-1 and T3SS-2) that promote transfer of bacterial proteins into host cells. We inactivated T3SS-1 (through a mutation in *invA*) and T3SS-2 (through a mutation in *spiB*), thus rendering *S. Typhimurium* unable to induce intestinal inflammation in the mouse colitis model (5, 6, 8). The *invA* and *spiB* mutations did not reduce the ability of *S. Typhimurium* to grow anaerobically on ethanolamine in vitro (Fig. 1C). Streptomycin-pretreated mice were inoculated with an equal mixture of an *invA spiB* mutant (SPN452) and an *invA spiB eutC* mutant (PT102). Mice infected with this mixture developed no signs of intestinal inflammation (Fig. 2 A–C). Equal numbers of both strains were recovered from colon contents (Fig. 2D and Fig. S1D). This finding demonstrated that in the absence of inflammation, ethanolamine did not promote luminal growth of *S. Typhimurium*.

**Effects of Inflammation on Ethanolamine Levels in the Gut.** We next investigated whether inflammation induces release of ethanolamine into the gut lumen because epithelial erosion is sometimes observed in the cecum during *S. Typhimurium* infection. To address this question, streptomycin-pretreated mice were orally inoculated with either the *S. Typhimurium* wild-type strain (IR715), or an *eutC* mutant (PT100) or an *invA spiB* mutant (SPN452). Colonic extracts of whole colons from these mice were analyzed for free ethanolamine by reverse-phase HPLC

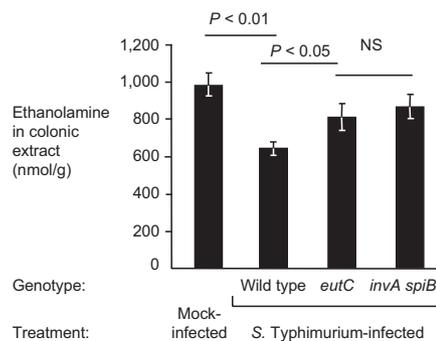


**Fig. 2.** Ethanolamine utilization genes confer a growth advantage in the inflamed intestine. (A–D) Streptomycin-pretreated mice (mouse colitis model) were orally inoculated with equal mixtures of *S. Typhimurium* strains [wild-type (IR715) vs. *eutC* mutant (PT100); *invA spiB* mutant (SPN452) vs. *invA spiB eutC* mutant (PT102); *ttrA* mutant (SW661) vs. *ttrA eutC* mutant (PT106)] as indicated below each panel. Samples were collected 4 d after infection. The number of animals is indicated in A. (A) Blinded, combined histopathology score of cecal inflammation showing averages (lines) of individual scores (circles). (B) Representative images of H&E-stained cecal sections. (Scale bars, 100  $\mu$ m.) (C) Expression of proinflammatory markers in the cecal mucosa. Relative mRNA levels of *Kc* (black) and *Nos2* (white) were determined by quantitative real-time RT-PCR analysis. Data shown are fold-changes over mock-treated control animals. (D) Competitive indices of *S. Typhimurium* strains recovered from the colon content. Data represent the geometric mean  $\pm$  SE. Statistical significance (*P* value) is indicated above the bars.

coupled with mass spectrometry (MS) (Fig. 3 and Fig. S6). Ethanolamine levels were assayed 4 d after infection. Mice infected with the fully virulent *eutC* mutant (PT100) showed colon ethanolamine levels that were similar to the colon levels in uninfected mice and to mice infected with a strain unable to cause inflammation (*invA spiB* mutant) (Fig. 3). This finding indicated that altered ethanolamine concentrations during inflammation were not the reason for the inflammation-dependence of the observed ethanolamine utilization phenotype.

**Ethanolamine Utilization Provides No in Vivo Growth Advantage in the Absence of Tetrathionate Respiration.** The *ttrA* gene encodes tetrathionate reductase subunit A and is essential for growth of *S. Typhimurium* on ethanolamine in vitro (Fig. 1C). To determine whether growth on ethanolamine provided an advantage in the absence of tetrathionate respiration in vivo, streptomycin-pretreated mice were infected with an equal mixture of a *ttrA* mutant (SW661) and a *ttrA eutC* mutant (PT106). Mice infected with this mixture developed acute intestinal inflammation (Fig. 2A–C and Fig. S2B). Both strains were recovered from colon contents in equal numbers (Fig. 2D), suggesting that even in the presence of inflammation, ethanolamine utilization did not provide a growth benefit without the ability to respire tetrathionate.

**Ethanolamine Utilization Increases Numbers of *S. Typhimurium* Cells Present in Inflamed Intestine.** To further investigate whether ethanolamine consumption contributes to intestinal colonization,



**Fig. 3.** Quantification of intestinal ethanolamine concentrations in the mouse colitis model. Streptomycin-pretreated mice were orally infected with the *S. Typhimurium* wild-type (IR715), an *eutC* mutant (PT100), an *invA spiB* mutant (SPN452), or mock-treated, as indicated below each panel. Four days after infection, the ethanolamine concentration in colonic extracts was determined by LC-MS/MS. Data represent the geometric mean of four animals  $\pm$  SE. Statistical significance (*P* value) is indicated above the bars.

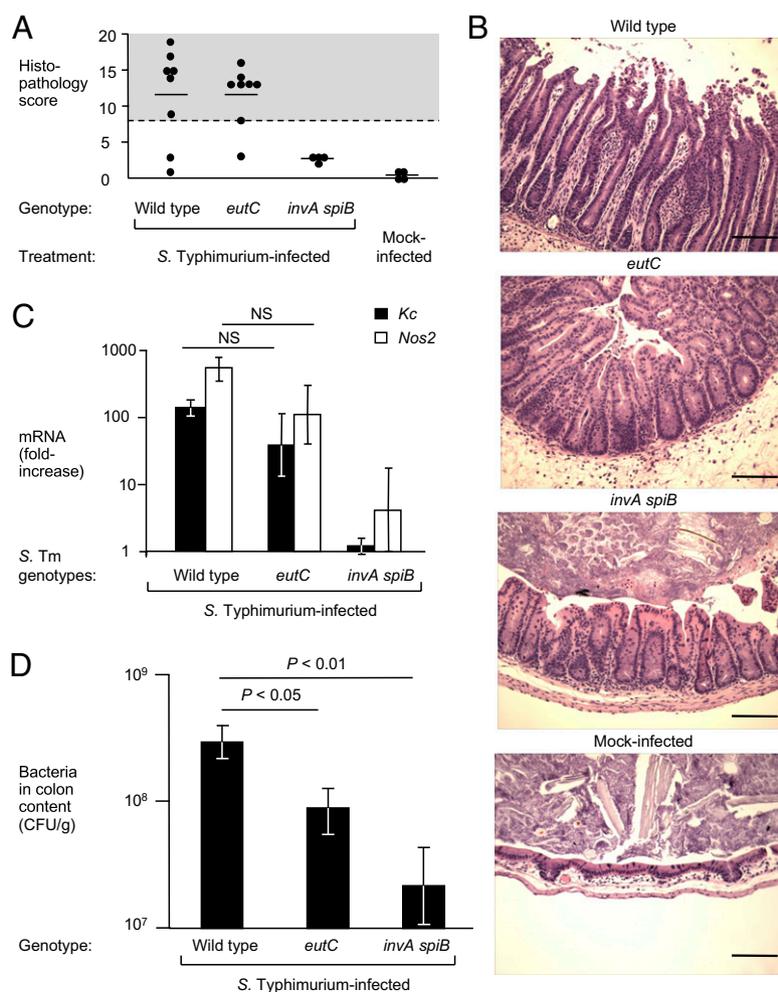
streptomycin pretreated mice were infected with individual *S. Typhimurium* strains. Mice were orally inoculated with the *S. Typhimurium* wild-type (IR715), an *eutC* mutant (PT100), or an *invA spiB* mutant (SPN452) and bacteria were recovered 4 d after infection (Fig. 4). Infection with the *S. Typhimurium* wild-type and the *eutC* mutant resulted in intestinal inflammation, but no marked inflammatory changes were observed in mice infected with the *invA spiB* mutant (Fig. 4 A–C). The *S. Typhimurium* wild-type was recovered in significantly ( $P < 0.05$ ) higher numbers from colon contents than the *eutC* mutant or the *invA spiB* mutant (Fig. 4D). Collectively, these data support the concept that ethanolamine utilization enhances the ability of *S. Typhimurium* to grow the lumen of the inflamed gut.

## Discussion

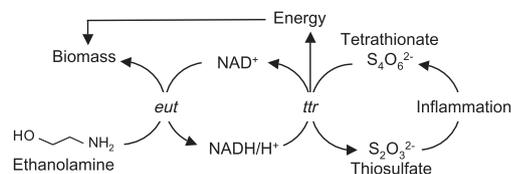
The principal strategy of *S. Typhimurium* for colonizing and disseminating through the intestinal route is to use its virulence factors (T3SS-1 and T3SS-2) to elicit acute intestinal inflammation (3). This host response stimulates oxidation of endogenous sulfur compounds (i.e., thiosulfate) and generates tetrathionate, which can be used as an electron acceptor that supports anaerobic respiration in *S. Typhimurium* (8) (Fig. 5). Here we show that

tetrathionate respiration in the inflamed intestine enabled *S. Typhimurium* to use ethanolamine, a nutrient that supports very little anaerobic growth by fermentation. Thus, by inducing intestinal inflammation, a pathogen may gain the ability to consume substrates (by respiration) that cannot be readily fermented. Through this mechanism pathogens can take advantage of the host inflammatory response to foster their own growth in the gut.

The ability to use specific, nonfermentable carbon sources may provide a substantial growth benefit in the inflamed intestine by reducing competition with microbes that rely entirely on a fermentative metabolism. The effectiveness of the strategy is illustrated by the finding that tetrathionate respiration enables *S. Typhimurium* to outgrow the microbiota in the inflamed intestine (8). Here we show that this growth relies at least in part on the ability to use the nonfermentable carbon source ethanolamine. The resulting outgrowth contributes to the long-term success of *S. Typhimurium* by enhancing its transmission to the next host by fecal shedding (10). The concept that intestinal inflammation empowers facultative anaerobic bacteria to sidestep nutritional competition in the gut might be applicable to a wide range of other host microbe interactions and to therapeutic strategies.



**Fig. 4.** Contribution of ethanolamine utilization to intestinal colonization in the mouse colitis model. (A–D) Streptomycin-pretreated mice were orally inoculated with the *S. Typhimurium* wild-type (IR715), an *eutC* mutant (PT100), an *invA spiB* mutant (SPN452), or mock-treated, as indicated below each panel. Samples were collected 4 d after infection. The number of animals is indicated in A. (A) Blinded histopathology scoring of cecal inflammation showing averages (lines) of individual scores (circles). (B) Representative images of H&E-stained cecal sections. (Scale bars, 100  $\mu$ m.) (C) Expression of proinflammatory markers in the cecal mucosa. Relative mRNA expression of *Kc* (black) and *Nos2* (white) was determined by quantitative real-time RT-PCR analysis. Data shown are fold-changes over mock-treated animals. (D) Recovery of *S. Typhimurium* from the colon content of infected mice. Data represent the geometric mean  $\pm$  SE. Statistical significance ( $P$  value) is indicated above the bars.



**Fig. 5.** Schematic of ethanolamine utilization and tetrathionate respiration in the inflamed gut.

## Materials and Methods

**Bacterial Strains.** The *S. Typhimurium* strains used in this study are listed in Table S1. Unless stated otherwise, bacteria were grown aerobically at 37 °C in Luria-Bertani (LB) broth (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) or on LB agar (15 g/L agar). When appropriate, antibiotics were added at the following concentrations: carbenicillin (0.1 mg/mL), chloramphenicol (0.03 mg/mL), nalidixic acid (0.05 mg/mL), kanamycin (0.05 mg/mL), and tetracycline (0.01 mg/mL).

**Construction of *S. Typhimurium* Mutants.** Standard cloning techniques were performed as described previously (19). P22 HT105 *int-201* generalized phage transduction was performed as describe previously (20). Plasmids and oligonucleotide sequences used in this study are listed in Tables S1 and S2, respectively. An internal fragment of the *eutC* gene was PCR amplified from the *S. Typhimurium* wild-type strain IR715 and cloned into pCR2.1 (Invitrogen) to generate pPT3. The “*eutC*” fragment from pPT3 was cloned into plasmid pEP185.2 to give rise to pPT5. Plasmid pPT5 was introduced into IR715 by conjugation to yield strain PT100. Strain PT100 was transduced to *eut*<sup>+</sup> (PT238), which removed the *eutC* insertion mutation. For complementation, a *his* mutation (*hisG::Tn10dTc*) was added to the *eutC* mutant (PT100) and a low-copy *F'* *his*<sup>+</sup> *eut*<sup>+</sup> plasmid (*F'* 606) was introduced by conjugational transfer selecting for *his*<sup>+</sup> (yielding strain TT26356). As a control, the *eutC::pPT5* mutation was introduced into plasmid *F'* 606 to give rise to *F'* pPT5. Plasmid *F'* pPT5 was introduced into PT100 by conjugational transfer selecting for *his*<sup>+</sup> to give rise to strain TT26358. A *phoN* mutation was introduced into TT26356 by transduction (yielding strain PT251) to distinguish the strain from TT26358 during competitive infection experiments. An *eutC::FRT* deletion was added to the chromosome of strain IR715 by first introducing  $\Delta 763$  (*cysA-eutS*::*MudA*) by transduction to yield strain TT26354. Strain TT26354 was then used as recipient in a transduction cross with a *cysA*<sup>+</sup> donor carrying the in-frame deletion mutation *eutC::FRT* to yield strain TT26355.

**In Vitro Anaerobic Growth Assays.** Three milliliters of ethanolamine broth [5 mM ethanolamine (Sigma-Aldrich) in No-carbon-E medium (21) supplemented with trace elements (17) and 40 mM sodium tetrathionate, as indicated] was inoculated with  $1 \times 10^8$  CFU/mL of each strain and anaerobically incubated at 37 °C (Bactron I anaerobic chamber; Sheldon Manufacturing). At the indicated time points, dilutions of the bacterial cultures were plated on LB agar plates containing the appropriate antibiotics.

**Animal Experiments.** All experiments in this study were approved by the Institutional Animal Care and Use Committee at the University of California at Davis.

**Mouse typhoid fever model.** Ten- to 12-wk-old female C57BL/6J wild-type mice were obtained from The Jackson Laboratory. Mice were inoculated intragastrically with  $5 \times 10^8$  CFU of the indicated bacterial strains. Bacterial numbers in the inoculum were determined by spreading serial 10-fold dilutions on agar plates containing the appropriate antibiotics. Animals were killed 4 d after infection. Tissue samples were weighed, homogenized in 5 mL PBS (T-25 homogenizer; IKA Works), and bacterial numbers enumerated by plating serial 10-fold dilutions of homogenates on selective media.

**Mouse colitis model.** C57BL/6J female wild-type mice and 129/svJ mice were obtained from The Jackson Laboratory. Ten- to 12-wk-old animals were pretreated with 20 mg streptomycin 24 h before infection. Mice were inoculated intragastrically with either 0.1 mL LB broth or *S. Typhimurium* strains (5). For competitive infections, mice were inoculated with an equal mixture of  $5 \times 10^8$  CFU of each strain. The ratio of recovered wild-type to mutant bacteria was normalized by the ratio in the inoculum to determine the competitive index. All animals were killed at the indicated time points and organs were collected for mRNA purification, histopathology analysis and to determine bacterial numbers, as described previously (22).

**Quantitative Real-Time RT-PCR Analysis.** Expression analysis was conducted as described previously (22). Briefly, RNA from mouse cecal tissues was isolated using TRI reagent (Molecular Research Center) according to the recommendations of the manufacturer. RNA was reversely transcribed using

TaqMan reverse transcription reagents (Applied Biosystems). Then, SYBR-Green (Applied Biosystems) based real-time PCR was performed using the primers listed in Table S2 and analyzed with comparative Ct method (Applied Biosystems). The levels of mRNA expression of target genes (*Kc* and *Nos2*) were normalized by the levels of *Gapdh* mRNA.

Bacterial RNA was isolated using the Aurum Total RNA Kit (Bio-Rad) according to the recommendations of the manufacturer. Afterward, an additional DNase treatment was performed using the Ambion DNA removal and inactivation kit. RT-PCR and real-time PCR was performed as described previously (23) with the following modifications: 0.3  $\mu$ g total RNA was used for reverse transcription and real-time PCR was performed using an ABI Vii7. The comparative CT method was used to analyze the data. Transcription of *eutL*, *eutK*, and *eutR* in each sample was normalized to the respective levels of guanyl nucleotide kinase mRNA, encoded by the *gmk* gene.

**Histopathology.** Formalin fixed, H&E-stained cecal tissue sections were blindly evaluated by an experienced veterinary pathologist. The scoring scheme is shown in Fig. S2A. Images were produced by an Olympus BX41 microscope.

**Measurement of Ethanolamine Concentrations in Intestine.** Ten- to 12-wk-old female wild-type mice (C57BL/6) were pretreated with 20 mg streptomycin for 24 h and orally inoculated with either 0.1 mL LB broth or  $1 \times 10^9$  CFU of the indicated bacterial strains. Four days after infection, a section of the colon was collected in 1 mL of ultrapure H<sub>2</sub>O (Invitrogen), weighed, and homogenized (T-25 homogenizer). Ethanolamine in the tissue homogenate was derivatized as described previously (14). Briefly, 1 mL of tissue homogenate was incubated with 100  $\mu$ L of 40% sulfosalicylic acid for 15 min then centrifuged at 11,000  $\times$  g for 15 min at room temperature. One milliliter of the supernatant was collected and 300  $\mu$ L of 0.5 M NaHCO<sub>3</sub>, 2 mL of fresh dansyl chloride solution [20 mg/mL of dansyl chloride (Sigma Aldrich) in acetone], and 200  $\mu$ L of 1 M NaOH was added. The samples were incubated for 20 min in the dark. Next, 200  $\mu$ L of 25% NH<sub>4</sub>OH was added to inactivate residual dansyl chloride. The volume was adjusted to 5 mL with acetonitrile. One milliliter of this sample was centrifuged at 11,000  $\times$  g for 1 min and the supernatant was used for further analysis. LC-MS/MS analysis of dansyl monoethanolamine was performed using a Surveyor HPLC separation module (ThermoFischer) coupled to a LTQ linear ion trap MS (ThermoFinnigan) equipped with APCI ion source. The sample tray compartment was kept at 15 °C. Ten milliliters of methanolic solution of dansyl monoethanolamine synthesized starting from monoethanolamine was injected onto a Chromolith Monolithic RP-18e C18 analytical column [100  $\times$  3 mm, (Merck)] equipped with a C18 guard cartridge. Mobile phases used were: A, 0.1% formic acid in deionized water and B, pure acetonitrile. Column temperature was 30 °C, and the flow rate was 0.4 mL/min. After a 0.2 min of isocratic run at 5% B, a gradient to 95% B was concluded at 6 min, and then continued at 5% B with to 0.5 min. The columns were equilibrated with the starting buffer for at least 3.5 min. The flow from the HPLC column was directed into the APCI ionization source of a LTQ linear ion trap MS (ThermoFinnigan) controlled with Xcalibur software (V1.4; ThermoFinnigan). Acquisition was performed in positive mode. Nitrogen sheath and auxiliary gas flow was 50 and 5 arbitrary units, respectively. Sweep gas flow rate was set to 5 units. Typical ion gauge pressure was 0.90\_10\_5. Vaporizer temperature was set to 450 °C and the discharge current was set to 5 mA. The capillary voltage was set to +9 V. Nitrogen sheath and auxiliary gas flow was 60 and 20 arbitrary units, respectively. The ion transfer capillary temperature was set to 275 °C. The AGC setting for selected ion monitoring and MS/MS was set to  $1 \times 10^3$ . Collision energy was set to 35%. A general MS/MS experiment was conducted to determine the fragmentation pattern (Fig. S3A), optimize the collision energy, and to choose the appropriate daughter ions for monitoring. Single ion monitoring scan spectra of parent ions were acquired from 292.5 to 297.5 at unit mass resolution with maximum injection time set to 200 ms in one micro-scan. The range of selected atomic mass units values corresponds to [M+H]<sup>+</sup> parent ion of dansyl monoethanolamine (Mi 295). SRM transitions were found 295/280, 295/171, and 295/157 (Fig. S3B). These transitions were used for dansyl monoethanolamine detection. The transition 295/280 generated the most intense daughter ion and was chosen for quantitation. The target was eluted at 1.79 min (Fig. S3C). HPLC grade acetonitrile was purchased from Burdick and Jackson (VWR International). Each lot of organic solvents was investigated by LC/MS infusion. Extra pure formic acid was purchased from EMD. The ultrapure water was supplied by an in-house Millipore system.

**Statistical Analysis.** Bacterial numbers, ethanolamine concentrations and changes in mRNA levels were transformed logarithmically before statistical analysis using a Student *t* test.

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