Host-mediated sugar oxidation promotes post-antibiotic pathogen expansion

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Changes in the gut microbiota may underpin many human diseases, but the mechanisms that are responsible for altering microbial communities remain poorly understood. Antibiotic usage elevates the risk of contracting gastroenteritis caused by *Salmonella enterica* serovars, increases the duration for which patients shed the pathogen in their faeces, and may on occasion produce a bacteriologic and symptomatic relapse. These antibiotic-induced changes in the gut microbiota can be studied in mice, in which the disruption of a balanced microbial community by treatment with the antibiotic streptomycin leads to an expansion of *S. enterica* serovars in the large bowel. However, the mechanisms by which streptomycin treatment drives an expansion of *S. enterica* serovars are not fully resolved. Here we show that host-mediated oxidation of galactose and glucose promotes post-antibiotic expansion of *S. enterica* serovar Typhimurium (*S. Typhimurium*). By elevating the expression of the gene encoding inducible nitric oxide synthase (iNOS) in the caecal mucosa, streptomycin treatment increased post-antibiotic availability of the oxidation products galactarate and glucarate in the murine caecum. *S. Typhimurium* used galactarate and glucarate within the gut lumen of streptomycin pre-treated mice, and genetic ablation of the respective catabolic pathways reduced *S. Typhimurium* competitiveness. Our results identify host-mediated oxidation of carbohydrates in the gut as a mechanism for post-antibiotic pathogen expansion.

A recent *in silico* analysis suggests that pathways involved in galactarate uptake and catabolism are associated with *S. enterica* serovars that cause gastrointestinal disease. Galactarate fermentation is one of the biochemical reactions used to differentiate members of the genus *Salmonella* into serovars. Although 98.2% of serovars associated with gastrointestinal infections can ferment this carbon source, only 15.4% of serovars associated with extraintestinal disease test positive for this reaction (Extended Data Fig. 1a). However, the biological significance of this association is not clear, because galactarate is a xenobiotic that is not normally produced by mammals or expected to be present within the diet. We therefore investigated the origin of galactarate in the intestine.

Consistent with the idea that galactarate is a xenobiotic, the concentration of this sugar in mouse chow was very low, as suggested by gas chromatography/mass spectrometry (GC/MS) measurements (Extended Data Fig. 1b). To investigate whether this nutrient is normally available to promote growth in mucus, we constructed a *S. Typhimurium* strain lacking the *gudT ygcY gudD STM2959* operon (*gudT–STM2959* mutant, Extended Data Fig. 1c), which encodes proteins involved in galactarate uptake and catabolism. Expression of the *gudT ygcY gudD STM2959* operon in *S. Typhimurium* is induced by hydrogen, a fermentation product of the gut microbiota. Deletion of galactarate utilization genes rendered *S. Typhimurium* unable to ferment galactarate and glucarate, but did not affect its ability to utilize other monosaccharides (Fig. 1a). Genetic ablation of the galactarate/glucarate utilization genes did not reduce the fitness of *S. Typhimurium* for anaerobic growth on hog mucin as the sole carbon source, but fitness of the *gudT–STM2959* mutant was reduced compared to the wild type when galactarate or glucarate was added to the medium (Fig. 1b). These data suggested that neither the diet nor the mucus naturally contained biologically relevant quantities of a substrate for enzymes encoded by the *gudT ygcY gudD STM2959* operon.

We next investigated the contribution of the *gudT ygcY gudD STM2959* operon to post-antibiotic pathogen expansion. Treatment of mice with a single dose of streptomycin one day before infection (pre-treatment with streptomycin) increased recovery of the wild-type *S. Typhimurium* from the colon contents of mice by approximately one order of magnitude compared to animals that had not received antibiotics (*P* < 0.05) (Fig. 1c). Genetic ablation of the galactarate/glucarate utilization genes significantly (*P* < 0.05) reduced recovery of *S. Typhimurium* from streptomycin pre-treated mice, but not from mice that had not received antibiotics. Genetic complementation with a plasmid carrying the cloned *gudT ygcY gudD STM2959* genes restored recovery of the *gudT–STM2959* mutant from streptomycin pre-treated mice to levels observed with the wild-type *S. Typhimurium*. Collectively, these data provided genetic evidence for a contribution of the *gudT ygcY gudD STM2959* operon to post-antibiotic expansion of *S. Typhimurium*.

Preconditioning of mice with streptomycin increases the severity of *S. Typhimurium* induced colitis. We therefore investigated whether the availability of galactarate and/or glucarate is elevated during severe colitis, a host response triggered by the action of two type III secretion systems (T3SS-1 and T3SS-2), which constitute the main virulence factors of *S. Typhimurium*. To prevent the generation of acute intestinal inflammation, we used avirulent *S. Typhimurium* strains lacking a functional T3SS-1 (due to a mutation in *invA*) and T3SS-2 (due to a mutation in *spiB*). Streptomycin pre-treated mice were infected either with a 1:1 mixture of the wild-type bacteria and a *gudT–STM2959* mutant or with a 1:1 mixture of an *invA spiB* mutant and an *invA spiB gudT–STM2959* mutant. In each competition, the galactarate/glucarate utilization-proficient strain (the wild-type bacteria or the *invA spiB* mutant) was recovered in higher numbers than the corresponding galactarate/glucarate utilization-deficient strain (the *gudT–STM2959* mutant or the *invA spiB gudT–STM2959* mutant) (Fig. 2a). However, only mice infected with a mixture of the wild-type bacteria and a *gudT–STM2959* mutant developed acute intestinal inflammation (Extended Data Fig. 2 and Extended Data Tables 1 and 2). When the experiment was repeated with mice that had not received streptomycin, the presence of genes for galactarate/glucarate utilization no longer conferred a fitness advantage (Fig. 2a). To distinguish between glucarate and galactarate as possible carbon sources, we inactivated *gudD*, encoding glucarate dehydratase, or *garD*, encoding galactarate dehydratase (Extended Data Fig. 1c). During *in vitro* growth, genetic ablation of *gudD* only reduced *S. Typhimurium* fitness in medium containing glucarate, while deletion of the *garD* gene only reduced fitness in...
medium containing galactarate (Extended Data Fig. 1d). Both the gudD gene and the gudT gene conferred a fitness advantage in streptomycin pre-treated mice (Extended Data Fig. 1e). Collectively, these data suggested that streptomycin treatment increased the availability of both glucarate and galactarate through a mechanism that was streptomycin-dependent, but independent of acute colitis triggered by S. Typhimurium virulence factors.

Antibiotic treatment increases the availability of sialic acid and fucose in the large intestine. It has been proposed that these monosaccharides are liberated by the resident microbiota from complex carbohydrates, a conclusion based on the observation that sialic acid and fucose are absent from caecal contents of germ-free mice. We thus investigated a conclusion based on the observation that sialic acid and fucose are absent from caecal contents of germ-free mice. We thus investigated whether the gut microbiota might play a role in liberating galactarate from complex carbohydrates in the intestine. Conventional mice (non-germ-free with a normal microbiota) received either streptomycin or vehicle control by oral gavage and the concentrations of galactarate and glucarate were measured in caecal contents by GC/MS four days later (Extended Data Fig. 3a, b). The concentration of galactarate was low in mice that had not received antibiotics, and streptomycin treatment resulted in a marked increase in the amount of galactarate present in the caecum (P < 0.001) (Fig. 2b). Similarly, streptomycin treatment increased (P < 0.001) caecal glucarate concentrations (Fig. 2c). We reasoned that if galactarate and glucarate present in streptomycin-treated mice was microbiota-liberated, then the concentrations of these sugars should be markedly reduced or absent in germ-free animals. Surprisingly, galactarate and glucarate levels measured by GC/MS in caecal contents of germ-free mice were similar or higher than those detected in conventional mice pre-treated with streptomycin (Fig. 2b, c). These data ruled out microbiota liberation as a possible mechanism by which streptomycin treatment elevated the availability of galactarate and glucarate in the murine large intestine.

Figure 2 | Post-antibiotic generation of galactarate and glucarate is Nos2-dependent. a, Groups of wild-type (C57BL/6) mice, streptomycin (Strep) pretreated C57BL/6 mice or streptomycin pre-treated Nos2-deficient (Nos2<sup>−/−</sup>) mice were infected either with a 1:1 mixture of the wild-type S. Typhimurium and a gudT<sup>−</sup>STM2959 mutant or with a 1:1 mixture of an invA spiB mutant and an invA spiB gudT<sup>−</sup>STM2959 mutant. Remarkably, in each experiment, the luminal content...
fitness advantage conferred by the galactarate/glucone utilization genes (Extended Data Fig. 2a). These data suggested that the Nos2 gene was necessary to generate a substrate for enzymes encoded by the gudT ygcY gudD STM2959 operon.

To determine whether the streptomycin-induced increase in the caecal galactarate and glucarate concentrations (Fig. 2b, c) was Nos2-dependent, we measured galactarate and glucarate concentrations in caecal contents from Nos2-deficient mice four days after streptomycin treatment by GC/MS. Strikingly, streptomycin treatment did not increase the availability of these sugars in Nos2-deficient mice (Fig. 2b, c). These data further supported the idea that generation of post-antibiotic galactarate and glucarate required an intact Nos2 gene.

To investigate whether the fitness advantage conferred by galactarate/glucone utilization required Nos activity, streptomycin-treated mice (C57BL/6) received drinking water supplemented with aminoguanidine hydrochloride, a specific iNOS inhibitor, and were infected with a 1:1 mixture of the wild-type S. Typhimurium and a gudT–STM2959 mutant. Inhibition of iNOS activity with AG significantly (P < 0.05) blunted the fitness advantage conferred by the galactarate/glucone utilization genes (Fig. 2a), suggesting that iNOS activity was required for generating galactarate and glucarate in the large intestine.

The host enzyme iNOS uses l-arginine to produce nitric oxide (NO), a reactive nitrogen species. Reactive nitrogen species are known catalysts in the oxidation of alcohols and aldehydes. We thus hypothesized that by generating reactive nitrogen species, streptomycin-induced iNOS synthesis might drive an oxidation of monosaccharides, thereby yielding the oxidation products galactarate and glucarate. To investigate whether reactive nitrogen species might oxidize galactose and glucose to galactarate and glucarate, respectively, we used 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), which is a stable free nitrosyl radical that mimics the activity of reactive nitrogen species (reviewed in ref. 16). Galactose and glucose were incubated in the presence of TEMPO and a co-oxidant (NaOCl) or the co-oxidant alone. Detection by GC/MS indicated that TEMPO oxidized galactose to galactarate (Fig. 2d) and glucose to glucarate (Fig. 2e). Next, we investigated whether the monosaccharides galactose and glucose were present in caecal contents. Galactose and glucose were detected in caecal contents of conventional (C57BL/6) mice, Nos2-deficient mice and germ-free mice (Fig. 2f, g). Collectively, these data suggested that monosaccharides were present in the murine caecum and could be oxidized by reactive nitrogen species to yield galactarate and glucarate.

Gene clusters for the utilization of galactarate and glucarate are also present in Escherichia coli and other related Enterobacteriaceae (Extended Data Fig. 1c). As treatment with streptomycin leads to an uncontrolled expansion of E. coli in the murine intestine, we investigated whether the underlying mechanism also involved utilization of galactarate and glucarate. To test this, we deleted the gudDXP and garD genes in the human E. coli isolate Nissle 1917 (Extended Data Fig. 1c). Deletion of the gudDXP and garD genes rendered E. coli unable to grow with galactarate or glucarate as the sole carbon source, but did not affect its ability to utilize glyceral (Extended Data Fig. 5a). The gudDXP and garD genes conferred a fitness advantage during growth of E. coli in the colon of streptomycin pre-treated mice, which was significantly (P < 0.05) diminished after treatment with the iNOS inhibitor aminoguanidine hydrochloride (Extended Data Fig. 5b).

Here we showed that by inducing the production of host-derived reactive nitrogen species, streptomycin treatment generates galactarate and glucarate in the gut lumen, thereby providing S. Typhimurium and E. coli with a considerable fitness advantage. Increases in galactarate and glucarate levels are also observed after treatment of mice with cefoperazone or a cocktail of vancomycin and bacitracin. A post-antibiotic expansion of Enterobacteriaceae is of concern due to the recent emergence of carbapenem antibiotic resistance within this group. Exposure of patients in intensive care units to broad-spectrum antibiotics is a known risk factor for acquiring an infection with carbapenem-resistant E. coli and Klebsiella isolates. Our findings identify host-mediated sugar oxidation as a new mechanism contributing to post-antibiotic expansion of Enterobacteriaceae.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Pavia, A. T. et al. Epidemiologic evidence that prior antimicrobial exposure decreases resistance to infection by antimicrobial-sensitive Salmonella. J. Infect. Dis. 161, 255–260 (1990).
2. Nelson, J. D., Kusmiesz, H., Jackson, L. H. & Woodman, E. Treatment of Salmonella gastroenteritis with ampicillin, amoxicillin, or placebo. Pediatrics 65, 1123–1130 (1980).
3. Akesson, B. & Bennett, J. V. Effect of antibiotic therapy in acute salmonellosis on the fecal excretion of salmonellae. N. Engl. J. Med. 281, 636–640 (1969).
4. Bohnhoff, M., Drake, B. L. & Miller, C. P. Effect of streptomycin on susceptibility of intestinal tract to experimental Salmonella infection. Proc. Soc. Exp. Biol. Med. 186, 132–137 (1954).
5. Nuccio, S. P. & Bäumler, A. J. Comparative analysis of Salmonella genomes identifies a metabolic network for escalating growth in the inflamed gut. MBio 5, e00929–14 (2014).
6. Kelterborn, E. Kaufmann–White–Schaef (1989) 1–171 (Bundesgesundheitsamt, 1992).
7. Lamichhane-Khadka, R., Frye, J. G., Porwollik, S., McClelland, M. & Maier, R. J. Hydrogen-stimulated carbon oxidation and conservation in Salmonella enterica serovar Typhimurium. J. Bacteriol. 193, 5824–5832 (2011).
8. Lamichhane-Khadka, R., Benoît, S. L., Maier, S. E. & Maier, R. J. A link between gut community metabolism and pathogenesis: molecular hydrogen-stimulated glucarate catabolism aids Salmonella virulence. Open Biol. 3, 130146 (2013).
9. Barthel, M. et al. Pretreatment of mice with streptomycin provides a Salmonella enterica serovar Typhimurium colitis model that allows analysis of both pathogen and host. Infect. Immun. 71, 2839–2850 (2003).
10. Tsolis, R. M., Adams, L. G., Ficht, T. A. & Bäumler, A. J. Contribution of Salmonella enterica virulence factors to diarrheal disease in calves. Infect. Immun. 67, 4879–4885 (1999).
11. Ng, K. M. et al. Microbiota-modulated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 502, 96–99 (2013).
12. Spees, A. M. et al. Streptomycin-induced inflammation enhances Escherichia coli gut colonization through nitrate respiration. MBio 4, e00430–13 (2013).
13. Stefanovic-Racic, M. et al. Comparison of the nitric oxide synthase inhibitors methyleneamine and aminoguanidine as prophylactic and therapeutic agents in rat adjuvant arthritis. J. Rheumatol. 22, 1922–1928 (1995).
14. Palmer, R. M., Ashton, D. S. & Moncada, S. Vascular endothelial cells synthesize nitric oxide from l-arginine. Nature 333, 654–666 (1988).
15. de Souza, M. The use of TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl) for the oxidation of primary and secondary alcohols. Quim. Nova 27, 287–292 (2004).
16. Sheldon, R. A. & Arends, I. W. C. E. Organocatalytic oxidations mediated by nitroxyl radicals. Adv. Synth. Catal. 346, 1051–1071 (2004).
17. Ozturk, A. & Freter, R. Ecological mechanism controlling growth of Escherichia coli in continuous flow cultures and in the mouse intestine. J. Infect. Dis. 114, 235–242 (1964).
18. Thenot, C. M. et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. Nature Commun. 5, 3114 (2014).
19. Hwang, I. et al. Alteration of gut microbiota by vancomycin and bacitracin improves insulin resistance via glucagon-like peptide 1 in diet-induced obesity. PLoS Pathog. 11, e1005135 (2015).
20. Tängdén, T. & Giske, C. G. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. J. Intern. Med. 277, 501–512 (2015).

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Author Contributions F.F. performed bacterial growth assays, most animal experiments and analysed the results. O.F. performed GC/MS measurements. M.X.B. scored histological sections. A.J.B., LT, C.A.L., E.M.V., T.K. and T.W. assisted with animal experiments. F.F., S.-P.N., R.M.T. and A.J.B. were responsible for the overall study design. F.F. and A.J.B. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.J.B. (ajbaumler@ucdavis.edu).

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METHODS

Bacterial strains and growth conditions. S. Typhimurium and E. coli strains used in this study are listed in Extended Data Table 3. All cultures were routinely grown aerobically at 37 °C in either Luria–Bertani (LB) broth (10 g per litre tryptone, 5 g per litre yeast extract, 10 g per litre NaCl) or on LB agar plates (1.5% Difco agar) unless indicated otherwise. When necessary, antibiotics were added to the medium at the following concentrations: nalidixic acid (Nal) 50 mg per litre, kanamycin (Km) 100 mg per litre, chloramphenicol (Cm) 30 mg per litre, carbenicillin (Carb) 100 mg per litre, spectinomycin (Spc) 50 mg per litre, and ampicillin (Amp) 200 μg per ml.

Sugar fermentation assay. 5 ml of fermentation broth (peptone, 10 g per litre, bromothymol blue, 0.024% per litre; final pH 7.4 ± 0.1) supplemented with the indicated carbon source (galactarate, glucarate, glucose, galactose, mannose or rhamnose, 10 g per litre each) or the control broth (no sugar added) were inoculated with 10 μl of an overnight culture of each indicated S. Typhimurium strain and incubated statically at 37 °C for 24 h. Fermentation of the sugar in the broth is indicated by a colour change from blue to yellow.

Anaerobic growth assays. 10 ml of M9 minimal medium (7.5 g per litre NaHPO4·2H2O, 30 g per litre KH2PO4, 5 g per litre NaCl, 10 g per litre NH4Cl, 0.1 μM CaCl2, 1 mM MgSO4, 0.001% thiamine) supplemented with hog mucin (0.1% w/v) or galactarate (0.04% w/v when added as sole carbon source, 0.1% w/v and 0.01% w/v when added to mucin broth) were inoculated with 2 × 103 colony-forming units (CFU) of each strain and incubated anaerobically at 37 °C for 24 h inside an anaerobic chamber (Bactron 1 Anaerobic Chamber; Sheldon Manufacturing, Cornelius). Bacterial numbers were determined by plating serial tenfold dilutions onto LB agar containing the appropriate antibiotics. The ratios of recovered wild-type and mutant bacteria after 24 h were normalized to the ratio at 0 h to calculate the competitive index.

Construction of plasmids. Standard cloning techniques were used to generate the plasmids used in this study. All plasmids and primers used in this study are listed in Extended Data Tables 4 and 5. PCR products were confirmed by sequencing (SeqWright Fisher Scientific, Houston). Suicide plasmids were propagated in E. coli DH5α pir. Plasmid pFF35 was constructed by PCR amplifying a 5′ flanking fragment of gudT using primers 71 and 72 and a 3′ flanking fragment STM2959 using primers 73 and 74. The two PCR fragments were gel purified, digested with XbaI and ligated with T4 DNA ligase (NEB). The ligation mix served as a template for a PCR with primers 71 and 74 and the product was gel purified and cloned into pCR2.1 using the TOPO TA cloning kit (Invitrogen). The plasmid construct was confirmed by sequencing and designated pFF35. To generate a suicide plasmid for replacing the gudT gycY gudD STM2959 genes with a kanamycin (KASS) cassette, the insert of plasmid pFF35 was excised using BamHI and ligated into the BamHI site of the suicide plasmid pRDH10. The resulting plasmid was digested with XbaI and ligated with a KSAC cassette generated from pBS34 by digestion with XbaI. The resulting suicide plasmid was designated pFF57.

To construct plasmids pFF62 and pFF63, respectively, chromosomal regions upstream and downstream of gudD and gudT in IR715 were amplified by PCR and cloned into BamHI-digested pRDH10 using Gibson Assembly Master Mix (NEB). To construct plasmids pFF64 and pFF65, respectively, chromosomal regions upstream and downstream of the gudD operon and of gudD from E. coli Nissle 1917 were amplified by PCR and cloned into BamHI-digested pRDH10 using Gibson Assembly Master Mix (NEB).

For complementation of the gudT–STM2959 mutant, the gudT gycY gudD STM2959 operon including its promoter region was PCR amplified using primers 92 and 93 or 94 and 95. The two PCR fragments were gel purified and cloned into BamHI-digested pWSK29 using Gibson Assembly Master Mix (NEB). The complementation plasmid was verified by sequencing and designated pGUDT.

Construction of mutants in S. Typhimurium. All suicide plasmids were introduced into S. Typhimurium IR715 recipient strains by conjugation using E. coli S17-1 λpir. Exconjugants were selected on LB + + Cm to select for clones that had integrated the suicide plasmid. Subsequent suicide counter-selection was performed as published previously21. Strains that were sucrose resistant and Cm2 were verified by PCR.

Plasmid pFF57 was introduced into Ff77 and SPN487 to generate FF162 (gudT–STM2959::Km3) and FF217 (ΔinvA ΔspiB ΔgudT–STM2959::Km3), respectively. Plasmid pFF62 was introduced into AJB715 to generate FF64 (phoN::Km3 ΔinvA ΔspiB gudD). Plasmid pFF63 was introduced into AJB715 to generate strain FF461 (phoN::Km3 ΔinvA ΔspiB gudD).

Construction of mutants in E. coli Nissle 1917. Suicide plasmids were introduced into E. coli Nissle 1917 (pSW172) recipient strains by conjugation using E. coli S17-1 λpir as the donor strain. To ensure stable propagation of pSW172, all steps of the conjugation were performed at 30 °C. Exconjugants were selected on LB + Carb + Cm to select for clones that had integrated the suicide plasmid. Subsequent sucrose counter-selection was performed as published previously21. Strains that were sucrose resistant and Cm2 were verified by PCR. If appropriate,
Measurement of sugar concentrations by GC/MS. Measurements were done at the West Coast Metabolomics Center at UC Davis as previously described. 20 mg of each sample, with d-Glucose-C-d7 added as the internal standard, were extracted with 1 ml of a pre-chilled acetonitrile/isopropanol:water (3:3:2) mixture. 450μl aliquots of the supernatants were evaporated to dryness and subjected to a two-step derivatization using methoximation and trimethylsilylation. GC/MS analysis was performed using an Agilent 7890 Gas Chromatography system coupled to an Agilent 5977A Mass spectrometer. An Rtx-5Sil MS w/Integra-Guard column (30 m x 250μm i.d., Restek), chemically bonded with a 1.4-bis(dimethyl-silox)phenylene-dimethyl polysiloxane cross-linked stationary phase (0.25μm film thickness) was used to separate the derivatives. Helium was used as a carrier gas at a constant flow rate of 1.2 ml min⁻¹. The GC oven temperature was programmed to increase from 50°C to 325°C at a rate of 10°C min⁻¹. The temperatures of the injector, transfer line, electron impact (EI) ion source, and quadrupole were set to 250°C, 290°C, 230°C and 150°C, respectively. The mass spectrometer was set to scan at a sampling rate of 4 and data was collected in a full scan mode (m/z 50 to 600). For quantification of sugars in the samples, a 6 point calibration curve was prepared with d-Glucose-C-d7 as internal standard. Agilent Mass Hunter quant software was used for data analysis.

Statistical analysis. The fold-changes of ratios for bacterial numbers and mRNA levels, respectively, and values for sugar concentrations were logarithmically transformed for statistical analysis. An unpaired Student’s t-test was used to determine whether differences between groups were statistically significant (P < 0.05). Error bars indicate standard error of the mean (s.e.m).
Extended Data Figure 1 | See next page for caption.
Extended Data Figure 1 | Galactarate/glucarate fermentation by S. enterica. a, One of the biochemical reactions used in the Salmonella serotyping scheme by Kauffman and White is the ability to ferment galactarate\(^6\). We divided 1,367 S. enterica subspecies enterica serovars into two groups: those associated with extraintestinal disease (serovars Typhi, Paratyphi A, Paratyphi B, Paratyphi C, Sendai, Choleraesuis, Typhisuis, Dublin, Bovismorbi ficans, Abortusovis, Abortusequi, Gallinarum biovar Gallinarum and Galliunarum biovar Pullorum) and those associated with human gastroenteritis (the remaining 1,354 serovars). The bar graph shows the percentages of serovars in each group that are positive, negative, delayed or differing (some isolates within the serovar are positive while others are negative) for this reaction. b, Detection of galactarate in chow for conventional or germ-free mice using GC/MS (n = 4).

c, Schematic drawing of the two gene clusters encoding proteins involved in the degradation of glucarate and galactarate in S. Typhimurium (ATCC14028), E. coli (Nissle 1917) and Klebsiella oxytoca (KCTC1686). Arrows indicate genes. The bracket indicates the DNA region deleted in the indicated mutants. d, Minimal medium or mucin broth supplemented with the indicated carbon sources (0.1% w/v) was inoculated with a 1:1 mixture of the wild-type S. Typhimurium and indicated mutants. Competitive index (CI) recovered after 24 h incubation in an anaerobic chamber. e, Streptomycin-treated C57BL6 mice (n = 6) were infected with a 1:1 mixture of the indicated S. Typhimurium strains and the competitive index in colon contents determined 4 days after infection. Bars represent geometric means ± standard errors (d, e). A Student’s \(t\)-test was applied to determine statistical significance.
Extended Data Figure 2 | Evaluation of caecal inflammation in streptomycin-treated mice 4 days after S. Typhimurium infection. a, Streptomycin pre-treated mice were infected with the indicated strain mixtures and caecal histopathology was scored four days later for four mice per group. The criteria used for histopathology scoring are listed in Extended Data Table 4. Each bar represents data from an individual animal. b, Representative images of haematoxylin and eosin (H&E)-stained caecal sections scored in a, along with an image from a mock-infected mouse for comparison. All images were taken at the same magnification. m, mucosa; s, submucosa; ml, muscle layer; lu, lumen.
Extended Data Figure 3 | Detection of galactaric acid and glucaric acid by GC/MS. a, Representative GC elution profile of a caecal sample containing galactaric acid and glucaric acid (arrows). b, Representative single ion monitoring scan spectrum of galactaric acid and glucaric acid.
Extended Data Figure 4 | Elevated Nos2 expression leads to nitrosyl radical-mediated oxidation of galactose. a, Expression levels of Nos2 mRNA in RNA isolated from the caecal tip three days after mock-treatment (mock) or treatment of mice with streptomycin (Strep) was determined by quantitative real-time PCR. Bars represent geometric means ± standard errors. A Student’s t-test was applied to determine statistical significance. b, Schematic of the oxidation of galactose to galactarate by TEMPO. 2,2,6,6-tetramethyl piperidine-1-oxyl (TEMPO) is a stable free nitrosyl radical that can oxidize terminal alcohol and aldehyde groups to carboxyl groups. Consumption of TEMPO during the redox reaction is prevented by addition of a co-oxidant (NaOCl), which regenerates the nitrosyl radical.
Extended Data Figure 5 | Galactarate/glucarate fermentation by E. coli.

**a**, Minimal medium or mucin broth supplemented with the indicated carbon sources was inoculated with a 1:1 mixture of the E. coli wild type (wt) and a garDXP garD mutant. CI, competitive index recovered after 24 h incubation in an anaerobic chamber. Growth was verified with 3 biological replicates. **b**, Streptomycin-treated C57BL6 mice (n = 6) were infected with a 1:1 mixture of the indicated E. coli strains and received the iNOS inhibitor aminoguanidine (AG) or vehicle control. The competitive index in colon contents was determined four days after infection. Bars represent geometric means ± standard errors (**a**, **b**). A Student's t-test was applied to determine statistical significance.

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**E. coli** wt vs. **gudDXP garD** mutant

Galactarate: + - - - -
Glucarate: - + - - -
Glycerate: - - + - -

Minimal medium  |  Mucin broth

Strep: + +
AG: - +

P < 0.0001
P < 0.05
ns

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Extended Data Table 1 | Chart indicating scoring criteria for blinded examination of H&E-stained sections from the caecum

| Score | Submucosal edema | Epithelial damage | Exudate | PMN infiltration* | Mononuclear cell infiltrate* |
|-------|------------------|-------------------|---------|-------------------|-----------------------------|
| 0     | No changes       | No changes        | No changes | No changes (0-5)  | No changes (0-5)             |
| 1     | Detectable (<10%)| Desquamation      | Slight accumulation | 6-20 | 5-10 |
| 2     | Mild (10%-20%)   | Mild erosion      | Mild accumulation | 21-60 | 10-20 |
| 3     | Moderate (20%-40%)| Marked erosion   | Moderate accumulation | 60-100 | 20-40 |
| 4     | Marked (>40%)    | Ulceration        | Marked accumulation | >100 | >40 |

*Number of cells per high-magnification microscopic field.
Extended Data Table 2 | Blinded histopathology scoring scheme

| Combined score | Description       |
|----------------|-------------------|
| >8             | Severe inflammation|
| 6-8            | Moderate inflammation|
| 3-5            | Mild inflammation  |
| 0-2            | Normal            |
Extended Data Table 3 | Bacterial strains used in this study

| Designation | Genotype | Reference |
|-------------|----------|-----------|
| **E. coli** |          |           |
| TOP10       | F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 lacX74 recA1 araD139 Δ(ara - leu)7697 galU galK rpsL endA1 nupG | Invitrogen |
| DH5a λpir   | F- endA1 hsdR17 (r-m+) supE44 thi-1 recA1 gyrA relA1 Δ(lacZYA-argF)U189 Φ80lacZΔM15 λpir | Lab stock |
| S17-1λpir   | zxx::RP4 2-(TetR::Mu) (KmR::Tn7) λpir | Lab stock |
| Nissle 1917 | Wild-type strain (O6:K5:H1) | 24 |
| FF441       | Nissle 1917 gudDXP garD | This Study |

**Salmonella**

| Designation | Genotype | Reference |
|-------------|----------|-----------|
| ATCC 14028  | Wild-type isolate of *S. enterica* serovar Typhimurium | ATCC |
| IR715       | Nalidixic acid-resistant derivative of ATCC14028 | 26 |
| CS019       | ATCC 14028 phoN::Tn10d-Cam | 26 |
| SPN487      | IR715 ΔinvA ΔspiB | 27 |
| FF176       | IR715 phoN::Tn10d-Cam | This Study |
| AJB715      | IR715 phoN::KmR | 28 |
| FF183       | IR715 phoN::Tn10d-Cam ΔinvA ΔspiB | This Study |
| FF162       | IR715 gudTygcYgudDSTM2959::KmR | This Study |
| FF461       | IR715 phoN::KmR ΔinvA ΔspiB gudD | This Study |
| FF464       | IR715 phoN::KmR ΔinvA ΔspiB gudD | This Study |
| FF217       | IR715 ΔinvA ΔspiB ΔgudTygcYgudDSTM2959::KmR | This Study |

References 24–28 are cited in this table.
### Extended Data Table 4 | Plasmids used in this study

| Designation | Relevant Characteristics | Reference |
|-------------|--------------------------|-----------|
| pCR2.1      | Cloning vector, Carb<sup>R</sup>, Km<sup>R</sup> | Invitrogen |
| pRDH10      | ori(R6K) mobRP4 sacRB Tet<sup>R</sup> Cm<sup>R</sup> | Lab stock |
| pBS34       | pBluescript II KS+, KSAC cassette, Carb<sup>R</sup>, Km<sup>R</sup> | 29 |
| pWSK29      | Cloning vector, ori(pSC101) Carb<sup>R</sup> | Lab stock |
| pFF35       | 5′ and 3′ flanking regions of *gudTygcYgudDSTM2959* operon in pCR2.1, Carb<sup>R</sup>, Km<sup>R</sup> | This Study |
| pFF57       | KSAC cassette flanked by up-/downstream regions of the *gudTygcYgudDSTM2959* operon in pRDH10; Cm<sup>R</sup>, Km<sup>R</sup> | This Study |
| pSW172      | ori(R101) *repA101*ts Carb<sup>R</sup> | 30 |
| pCAL61      | ori(R101) Kan<sup>R</sup> Strep<sup>R</sup> | 12 |
| pCAL62      | ori(R101) Carb<sup>R</sup> Strep<sup>R</sup> | 12 |
| pFF62       | Up-/downstream regions of *gudD* from IR715 in pRDH10 | This Study |
| pFF63       | Up-/downstream regions of *garD* from IR715 in pRDH10 | This Study |
| pFF64       | Up-/downstream regions of the *gudDXP* operon from EcN in pRDH10 | This Study |
| pFF65       | Up-/downstream regions of *garD* from EcN in pRDH10 | This Study |
| pGUDT       | *gudTygcYgudDSTM2959* operon under the control of its native promoter in pWSK29; Carb<sup>R</sup> | This Study |

References 29 and 30 are cited in this table.
### Extended Data Table 5 | Primers used in this study

| Deletion of gudTygcYgudDSTM2959 | Primer | Sequence* |
|----------------------------------|--------|-----------|
| 71                               | 5'-GGATCCCTCTGAACCGCTGCTATGG-3' |
| 72                               | 5'-TCTAGAGTTACGCTGATTGATTTAG-3' |
| 73                               | 5'-TCTAGAGTATGGGAAATCAGAGATAACG-3' |
| 74                               | 5'-GGATCCAGGGAGATACGCATATGG-3' |

**Deletion of gudD in IR715**

| Primer | Sequence* |
|--------|-----------|
| 116    | CACACCGGTCCTGTGCTCAACATGCACGATTCG |
| 117    | CGATCTCCCGATTTACCATGTG |
| 118    | TAAACATCGGAGAGATCGAAGTTTGAAG |
| 119    | GCGTCCGGCCTAGAGGACTGCTTGAGGAAAG |

**Deletion of garD in IR715**

| Primer | Sequence* |
|--------|-----------|
| 112    | CACACCGGTCCTGTGCTGAAATCAGAATGGTC |
| 113    | TCACAGGTGCAGAATGTGTTACAGTCAGTTC |
| 114    | CATATTCCGAGCTGTGACCTGACTATTCTG |
| 115    | GCGTCCGGCCTAGAGGATTGCTGCAAGGCTTCAC |

**Complementation of gudTygcYgudDSTM2959**

| Primer | Sequence* |
|--------|-----------|
| 92     | 5'-TCCTGCAGCCCGGCTGCTGCTTGAGTACGAAGG-3' |
| 93     | 5'-TTAAGCACCAGCTCAAGGAC-3' |
| 94     | 5'-CCTGCAATGTTGCCAGTCATAGTC-3' |
| 95     | 5'-CGCTCTAGAATCATGCTCCGGCTACAACCACG-3' |

**Deletion of gudDXP operon in E. coli Nissle 1917**

| Primer | Sequence* |
|--------|-----------|
| 136    | CACACCCGTCCTGTGCTGCTGTTATGCGGATG |
| 137    | CCGGTTCGCCCTGTGCCGATGTAC |
| 138    | CCAGGGCAACGAGCAACGGCAATAGAAAAAG |
| 139    | GCGTCCGGGCTAGAGTGGCTGAGTCAGGCG |

**Deletion of garD in E. coli Nissle 1917**

| Primer | Sequence* |
|--------|-----------|
| 235    | CACACCCGATCGCTGCTGTGGAACATCAAATCAG |
| 236    | CACGCGTGGTACGCCAGTTAGGCTAG |
| 237    | ACCCGAACCACCCGTAACCTGATTTC |
| 238    | GCGTCCGGGCTAGAGGCGCGCAGAAAAGTTCTTTC |

**Quantitative real-time RT-PCR**

| Organism   | Target | Sequence |
|------------|--------|----------|
| Mus musculus | B2M    | 5'-GGTCTTTTCTGCTGCTTTCTCA-3' |
|             |        | 5'-GGTCTTTTCTGCTGCTTTTCC-3' |
| Mus musculus | Nos2   | 5'-TTGGGTTCCTTGGTCTCCACGG-3' |
|             |        | 5'-CCTCTTTTTCACTTCAGGTTGTAAG-3' |

*Restriction enzyme sites are underlined, overlapping sequences for Gibson Assembly are in bold.