Diet-derived galacturonic acid regulates virulence and intestinal colonization in enterohaemorrhagic Escherichia coli and Citrobacter rodentium

Angel G. Jimenez1,2, Melissa Ellermann1,2, Wade Abbott3,4 and Vanessa Sperandio1,2*

Enteric pathogens sense the complex chemistry within the gastrointestinal tract to efficiently compete with the resident microbiota and establish a colonization niche. Here, we show that enterohaemorrhagic Escherichia coli and Citrobacter rodentium, its surrogate in a mouse infection model, sense galacturonic acid to initiate a multi-layered program towards successful mammalian infection. Galacturonic acid utilization as a carbon source aids the initial pathogen expansion. The main source of galacturonic acid is dietary pectin, which is converted to galacturonic acid by the prominent member of the microbiota, Bacteroides thetaiotaomicron. This is regulated by the ExuR transcription factor. However, galacturonic acid is also sensed as a signal through ExuR to modulate the expression of the genes encoding a molecular syringe known as a type III secretion system, leading to infectious colitis and inflammation. Galacturonic acid acts as both a nutrient and a signal directing the exquisite microbiota-pathogen relationships within the gastrointestinal tract. This work highlights that differential dietary sugar availability influences the relationship between the microbiota and enteric pathogens, as well as disease outcomes.

The mammalian gastrointestinal tract is populated by a dense and highly adapted microbiota. Glycan foraging has a key role in the establishment and maintenance of these microbial communities. Bacterial pathogens have evolved metabolic adaptations and elaborate virulence mechanisms to effectively compete with commensal microbes. Enterohaemorrhagic Escherichia coli (EHEC) has a very low infectious dose, estimated to be 50 colony-forming units (c.f.u.). Given that EHEC colonizes the colon, one of the regions most heavily colonized by the microbiota, it must be an extremely efficient pathogen to gain a foothold in the gastrointestinal tract.

The microbiota poses a substantial barrier to enteric pathogens. EHEC and C. rodentium (a murine pathogen widely used as a surrogate model for EHEC infection) can overcome this barrier by deploying the type 3 secretion system (T3SS). The T3SS is a molecular syringe that many Gram-negative pathogens use to translocate their repertoire of effector proteins into host cells. These effectors either hijack or mimic eukaryotic cell function to benefit the pathogen. The genes encoding EHEC and C. rodentium T3SSs are contained in a pathogenicity island called the locus of enterocyte effacement (LEE). Expression of the LEE in C. rodentium is downregulated by 21 d after infection, and the pathogen is quickly outcompeted by the microbiota and cleared from the intestine. The ability of the microbiota to outcompete C. rodentium is dependent on nutrient availability. Saccharolytic members of the microbiota, such as Bacteroides thetaiotaomicron (Bt), which can break down and utilize complex carbohydrates, do not compete with C. rodentium for nutrients. However, commensal E. coli, which can only utilize mono- and disaccharides, are direct competitors, resulting in the ultimate clearance of the pathogen.

EHEC and commensal E. coli strains utilize overlapping and distinct sugars to establish and maintain host colonization. Notably, catabolism of sucrose and galacturonic and glucuronic acids but not gluconate is important for colonization by EHEC, whereas commensal E. coli strains use gluconate but not galacturonic and glucuronic acids, suggesting that EHEC has evolved different nutritional requirements than the closely related commensal E. coli. Galacturonic acid is a dietary-derived metabolite that is necessary for the establishment of pathogenic strains of E. coli in the gut. EHEC metabolizes this sugar using the Ashwell pathway, which is under the control of the ExuR transcriptional regulator. A high throughput screen for metabolic pathways and transcriptional factors that regulate LEE gene expression has identified ExuR as a regulator of LEE. ExuR is a member of the GntR family of transcriptional regulators, has been previously characterized as a regulator of sugar acid catabolism in E. coli and is responsive to galacturonic acid.

Here, we show that the ExuR regulon comprises the galacturonic acid utilization genes and the LEE. We show that galacturonic acid utilization as a carbon source aids pathogen expansion only at the beginning of infection. Moreover, as infection proceeds, ExuR, in the absence of galacturonic acid, acts as an activator of the LEE genes leading to colitis and inflammation. In sum, ExuR orchestrates enteric pathogenesis by shifting its role as a regulator of metabolism and virulence in the context of microbiota and diet.

**Results**

**ExuR directly regulates LEE genes.** A screen to identify genes that influence expression of LEE genes has identified ExuR as a LEE regulator. ExuR is a transcription factor that regulates sugar acid metabolism in E. coli. To assess whether the Ashwell pathway is regulated by ExuR in EHEC and to corroborate the findings of our screen, we generated an isogenic ΔexuR EHEC mutant. Transcriptomic (European Nucleotide Archive accession number: PRJEB30676) and targeted quantitative PCR with reverse transcription (RT-qPCR) analysis confirmed that ExuR regulates
Fig. 1 | ExuR activates expression of the LEE in EHEC. a, Heat map depicting the top changed transcripts between WT and ΔexuR EHEC. b, Schematic representation of the LEE-encoded T3SS and the LEE island. CR, C. rodentium. c–e, RT-qPCR of LEE-encoded genes ler (c), espA (d) and tir (e) in WT (n = 12), ΔexuR (n = 12) and complemented (p-exuR) (n = 6) (n, number of biological replicates; results are representative of three independent experiments). Fold change relative to p0A as an internal control. Shown are mean and P values (two-sided unpaired Mann–Whitney test). f, Western blot for LEE-encoded proteins Tir, EspB and EspA in secreted and lysate fractions. Total proteins are used as loading control for whole-cell lysates. Cells were collected in late logarithmic phase at the same OD600, and supernatants were concentrated for secreted proteins. Bovine serum albumin (BSA) is used as a loading control. Shown are mean and s.d. from three independent experiments. g, ExuR consensus sequence. h, Sequence of the ler probe used for EMSAs depicting the location of the ExuR consensus sequence (highlighted in yellow), promoters P1 (red) and P2 (pink). i, EMSA assay for the ler putative ExuR-binding site, a known target of ExuR, and the kanamycin probe as a negative control. Results are representative of three independent experiments. j, Fluorescein actin staining analysis. HeLa cells were infected with mCherry-expressing WT (n = 100), ΔexuR (n = 100) and complemented (p-exuR) (n = 100) strains of EHEC (n, number of infected HeLa cells enumerated for number of pedestals; results are representative of three independent experiments). Data are mean ± s.d. from three independent experiments. P values were determined by one-way analysis of variance (ANOVA). k, Representative confocal microscopy images of pedestal formation (white arrows) by mCherry-expressing EHEC. DNA (blue) is stained with 4,6-diamidino-2-phenylindole and actin (green) is stained with fluorescein isothiocyanate (FITC)-phalloidin. Images at original magnification, ×40; scale bars, 20 μm.

the Ashwell pathway and expression at the LEE in EHEC (Fig. 1a and Extended Data Fig. 1). Deletion of exuR led to a significant decrease in transcript levels of LEE-encoded genes (ler, espA and tir) (Fig. 1c–e) and proteins in EHEC grown in Dulbecco’s modified Eagle medium (DMEM) in the absence of galacturonic acid (Fig. 1f). An in silico search for the ExuR DNA-binding consensus sequence14 (Fig. 1g) revealed a potential binding site in the regulatory region of the ler gene. The LEE harbours 41 genes, the majority of which are organized in five operons, named LEE1–LEE5. Ler is encoded by the first gene in the LEE1 operon, and is the activator of all of the LEE genes10 (Fig. 1b). EHEC LEE1 has two promoters, P1 and P214, and the ExuR DNA-binding consensus sequence is upstream of P1 (Fig. 1b). ExuR directly binds to the ler regulatory region (Fig. 1b and Extended Data Fig. 1c) to activate ler transcription, and consequently LEE-gene expression (Fig. 1c–e). The LEE-encoded T3SS promotes the formation of attaching and effacing lesions on epithelial cells. These lesions lead to effacement of the microvilli and rearrangement of the host cell actin, forming pedestal-like structures below the attached bacteria1. Congruent with the decreased expression of LEE genes, ΔexuR forms fewer attaching and effacing lesions than the wild-type (WT) and complemented strains (Fig. 1j,k).
Sensing of galacturonic acid decreases expression of the T3SS and is dependent on ExuR. *E. coli* can transport and utilize galacturonic and glucuronic acids as carbon sources\(^{19}\). ExuR controls the transport and catabolism of sugar acids (via *uxaABC*, *uxuAB*, *uxuR* and *exuT* genes). In the absence of galacturonic acid, ExuR represses transcription of the *uxaAC*, *uxuAB*, *uxuR* and *exuT* genes and activates LEE gene expression\(^{5,10}\) (Fig. 1a and Extended Data Fig. 1a). Galacturonic acid binds to ExuR and hinders its ability to interact with DNA\(^{5,10}\). Consequently, in the presence of galacturonic acid, the genes necessary for its utilization are expressed\(^{5,10}\) and the LEE genes are repressed (Fig. 2a–c). Notably, repression of the LEE by galacturonic acid is dependent on ExuR (Fig. 2d,e). The LEE is also present in enteropathogenic *E. coli* (EPEC), in which its repression is similar to EHEC\(^{7}\). Consequently, galacturonic acid also represses LEE gene expression in EPEC (Extended Data Fig. 3d). ΔuxaC EHEC (UxaC encodes the first enzyme in galacturonic acid utilization) cannot grow in minimal media when glucuronic or galacturonic acid is a sole carbon source (Extended Data Fig. 2a). However, it can grow in low-glucose DMEM in the absence or presence of galacturonic acid (Extended Data Fig. 2a,c). Growth of EHEC in low-glucose DMEM promotes LEE gene expression in vitro\(^{5,10}\). To probe whether LEE gene regulation by galacturonic acid is dependent on it being utilized as a carbon source or as a signal, we titrated the concentration of this sugar to levels at which it was no longer used for growth (Extended Data Fig. 2c). At a concentration of 100 µM, galacturonic acid does not contribute to growth of EHEC (Extended Data Fig. 2c), but still serves as a signal to decrease LEE gene expression (Fig. 2e). These findings suggest that galacturonic acid serves as both a nutrient and a signal to modulate gene expression.

**ExuR is important for *C. rodentium* murine pathogenesis.** EHEC is a human pathogen and therefore murine models of EHEC infection fail to recapitulate several of the key features of EHEC pathogenesis. These include attaching and effacing lesion formation, induction of inflammation and mucosal colonization. *C. rodentium* has been used extensively as a model of EHEC infection\(^{15}\). *C. rodentium* also harbours the LEE and forms attaching and effacing lesions in the colon of mice\(^{23}\). Notably, LEE regulation by ExuR is important for murine pathogenesis. EHEC (Extended Data Fig. 3a,b). To investigate the role of ExuR during mouse infection, C3H/HeJ mice (this strain of mice, in which infection is fatal, is more susceptible to *C. rodentium* infection) were orally inoculated with WT *C. rodentium* DBS100 or with a ΔexuR isogenic mutant. Mice that were...
infected with WT *C. rodentium* completely succumbed to the infection by day 11. However, mice that were infected with the ΔexuR mutant survived (Fig. 3a). Deletion of *exuR* also affected colonization of mice, leading to reduced *C. rodentium* loads in stools and caecal content compared with mice infected with WT *C. rodentium* (Fig. 3b–d). The ΔexuR *C. rodentium* failed to colonize the caecal and colonic tissue (Fig. 3c,e). Tissue colonization of *C. rodentium* is strictly dependent on the LEE-encoded T3SS. Congruent with the marked reduction in transcript levels of LEE-encoded genes in vitro (Extended Data Fig. 3b), transcript levels of the LEE-encoded genes *ler*, *espA* and *tit* in mice infected with the ΔexuR mutant were lower compared with mice infected with WT *C. rodentium* (Fig. 3f–h). Pathology and expression of pro-inflammatory cytokines (Nos2 and Il22) were also decreased in animals infected with ΔexuR (Fig. 3i–l). Histological damage in WT infected mice was characterized by the presence of oedema, the noticeable loss of epithelial integrity, moderate to severe crypt hyperplasia, loss of goblet cells, immune cell infiltration into the lamina propria and submucosa and transepithelial migration of immune cells into the lumen (Fig. 3i,l). By contrast, histopathology scores in mice infected with the ΔexuR mutant were similar to those of uninfected controls. Collectively, colitis severity corresponded with a decreased ability of the ΔexuR mutant to establish successful infection in the mouse colon and a decreased lethality compared with the parental DBS100 strain.

Because ExuR activates expression of the T3SS (Fig. 1), which leads to induction of inflammation in the gut\(^1\), we investigated whether ExuR was necessary for *C. rodentium* colonization after inflammation occurred. To induce inflammation, we used chemical induction of colitis by treatment with dextran sodium sulfate (DSS). DSS directly damages the colonic epithelium and induces severe colitis in the absence of any bacterial pathogen\(^1\). DSS treatment sensitized mice to such a degree that they became susceptible to infection by ΔexuR *C. rodentium* (Fig. 4). The ΔexuR *C. rodentium* colonized mice to a similar degree to WT *C. rodentium* (Fig. 4a), and had higher bacterial loads in the caecal content and tissue of DSS-treated mice compared with non-infected animals (Fig. 4b,c). Consistent with the rescue of tissue colonization with DSS treatment, ΔexuR *C. rodentium* infection was fatal to DSS-treated mice (Fig. 4d). Together, these data show that ExuR is not essential for survival in an inflamed gut, and that its principal role is the induction of inflammation via regulation of the LEE.

**Microbiota–pathogen relationships in the catabolism of sugar acids for intestinal colonization by *C. rodentium*.** To better understand the role of sugar acid catabolism in intestinal colonization by *C. rodentium*, we generated a ΔuxuR mutant. The *uxu* gene encodes a uronate isomerase, which, in the Ashwell pathway, is the first step in the catabolic pathway for galacturonic acid and glucuronic acid utilization. UxaC converts galacturonic acid to tagaturonate, which is then converted by UxaB to altronate. UxaC converts glucuronic acid to tagaturonate, which in the catabolic pathway for galacturonic acid and glucuronic acid utilization, is a uronate isomerase, which, in the Ashwell pathway, is the first step of metabolism of sugar acids. These data suggest that catabolism of sugar acids has a role in initial intestinal expansion by *C. rodentium*, but is not necessary during later stages of infection (Fig. 5b).

To further probe the role of ExuR regulation of the Ashwell pathway and LEE gene expression during mouse infection, we infected mice with WT, ΔuxuR, ΔuxuC and ΔuxuCΔuxuR (ΔΔ) mutant *C. rodentium*, and determined the bacterial burden and mouse survival (Fig. 5e–g). Mice that were infected with the double mutant were not significantly different from the single ΔexuR mutant in relation to stool and tissue bacterial loads, pathology scores (Extended Data Fig. 5) or survival (Fig. 5e–g). Although ΔuxuCΔuxuR *C. rodentium* presents decreased stool burdens compared to the WT up to 2 d after infection (Fig. 5b,e), it does not have a defect in caecum tissue colonization, which is an LEE-dependent phenotype (Fig. 5f).

Moreover, the ΔuxuC-infected mice succumb similarly to mice infected with WT *C. rodentium*, and have increased pathology compared with ΔexuR and ΔuxuCΔuxuR (Fig. 5g and Extended Data Fig. 5). Competition experiments show that WT *C. rodentium* outcompetes ΔexuR and ΔuxuRΔuxuC *C. rodentium* by 1,000-fold, whereas ΔuxuC is outcompeted by WT by tenfold (Fig. 5h). This indicates that colonization of the gut is mainly accomplished by LEE activation through the ExuR transcriptional regulator. Furthermore, the single ΔuxuC mutant outcompetes the ΔuxuRAuxuC mutant, indicating that deletion of *exuR* is detrimental towards colonization even in the absence of sugar acid catabolism (Fig. 5h). UxaC acts on both glucuronic and galacturonic acid; therefore, changes in mouse colonization by ΔuxuC is representative of the overall role of sugar acid utilization, without discriminating between the two branches of the Ashwell pathway. To address this, we also independently deleted the *C. rodentium* *uxaB* and *uxuB* genes and performed further competition experiments in vivo. The ΔuxaB mutant exhibits a similar defect to ΔuxuC *C. rodentium*, whereas the ΔuxuB mutant did not exhibit a defective phenotype (Fig. 5h). Overall, this suggests that galacturonic acid—and not glucuronic acid—provides the initial fitness advantage for *C. rodentium*.

The T3SS induces inflammation in the gut\(^1\). In an already inflamed gut (caused by DSS treatment), mice succumb to infection by ΔexuR *C. rodentium* (Fig. 4); congruent with these data, ΔexuR *C. rodentium* is not outcompeted by the WT in these animals (Fig. 5h). However, ΔuxuCΔuxuR *C. rodentium* is similarly outcompeted by the WT in the absence or presence of inflammation (Fig. 5h). Thus, catabolism of sugar acids does not have a substantial role in the regulation of the T3SS in vivo, indicating that regulation of the T3SS through ExuR is independent of its role in regulation of metabolism of sugar acids. These data suggest that sugar acid utilization has a role in early pathogen expansion but is not involved in virulence.

The main source of pectin in the gastrointestinal tract is diet. Pectin is a polysaccharide found in plant cell walls\(^27\) that can be
Fig. 3 | Deletion of exuR results in decreased morbidity and colonization during mouse infection by C. rodentium. a, Survival curves for C3H/HeJ mice infected with WT (n = 12) or ΔexuR (n = 12) C. rodentium. PBS (n = 8) was used as a negative control. Two-sided log-rank (Mantel–Cox) test. b, Bacterial c.f.u. in stools of C3H/HeJ mice infected with WT (n = 12) or ΔexuR (n = 12) C. rodentium. c–e, Measurement of bacterial c.f.u. from C3H/HeJ mice infected with WT (n = 12) or ΔexuR (n = 12) C. rodentium to assess bacterial abundance from the lumen and epithelium in the caecum and colon at 8 d post-infection (peak of disease). C. rodentium c.f.u. from colon tissue (c), caecal content (d) and caecal tissue (e). In b–e, n is number of biological replicates; results are representative of three independent experiments. Shown are mean and P values (two-sided unpaired Mann–Whitney test). Dashed lines are representative of the limit of detection for the assay. f–h, RT-qPCR of Lee-encoded genes ler (f), espA (g), and tir (h) from the caecal content of mice infected with WT (n = 8) or ΔexuR (n = 8) C. rodentium, collected 8 d post-infection (n, number of biological replicates; results are representative of two independent experiments). Shown are mean and P values (two-sided unpaired Mann–Whitney test). i, Blinded histopathology scores of non-infected mice or mice infected with WT (n = 8) or ΔexuR (n = 8) C. rodentium (n, number of biological replicates; results are representative of two independent experiments). Shown are mean and P values (two-sided one-way ANOVA). j, k, RT-qPCR of expression of Nos2 and Il22 in the caecal tissue of C3H/HeJ mice after infection with WT (n = 8) or ΔexuR (n = 8) C. rodentium (n, number of biological replicates; results are representative of two independent experiments). Shown are mean and P values (two-sided unpaired Kruskal–Wallis test). Expression was normalized to GpdpH. PMN, polymorphonuclear leukocyte; MC, monocytes; I, Haematoxylin and eosin-stained colon tissues of C3H/HeJ non-infected mice or mice infected with WT or ΔexuR C. rodentium at 8 d post-infection. Representative images from two independent experiments. Scale bars, 100 μm.
digested—releasing galacturonic acid—by saccharolytic members of the microbiota, such as *Bt*. In the presence of galacturonic acid, ExuR repression of the genes encoding enzymes involved in its utilization is released, whereas LEE gene expression is decreased (Fig. 2). Consistent with this regulation, expression of *uxuA* and *uxuC* is increased in the presence of *Bt* when pectin is present (Fig. 6b,c). Expression of the LEE gene espA is decreased in the presence of *Bt* when pectin is present (Fig. 6a). However, espA expression is increased in the presence of *Bt* in the absence of pectin (Fig. 6a), due to the production of succinate by *Bt*, as previously described. *Bt* can derive several sugars from mucin and digest pectin, releasing galacturonic acid. We used supernatants of *Bt* grown in a defined medium with either mucin or pectin as carbon sources in in vitro competition experiments between WT and ΔuxuA *C. rodentium* (which cannot utilize galacturonic acid as a sugar source) to investigate whether galacturonic acid utilization conferred a growth advantage to *C. rodentium* in relation to other sugar sources. The WT *C. rodentium* outcompeted ΔuxuA tenfold in mucin-pre-conditioned media, whereas it outcompeted ΔuxuC *C. rodentium* 100-fold in pectin-pre-conditioned media (Fig. 6d). Notably, there were no differences in the competitive index between WT and ΔuxuA *C. rodentium* under growth on mannose or starch, which are substrates that do not contain uronic acids (Fig. 6d). These data indicate that *Bt* releases galacturonic acid from pectin, increasing expression of the galacturonic acid utilization genes, leading to a growth expansion of *C. rodentium*, whereas expression of the T3SS is decreased.

To address the role of pectin in *C. rodentium* mouse colonization and infection, mice were either fed pectin or not. However, we observed that C3H/HeJ mice that were fed pectin presented basal levels of inflammation compared with mice that were not fed pectin (Extended Data Fig. 6). To avoid these issues, we used C57BL/6 mice—in which pectin does not promote inflammation—which also develop colitis and disease on *C. rodentium* infection, but in which infection is not fatal. *C. rodentium* loads were similar in the caecal tissue of mice treated with WT or ΔexuR *C. rodentium* (Fig. 6e). However, *C. rodentium* loads were decreased in the caecal tissue of mice treated with pectin (Fig. 6f). Attachment to *C. rodentium* to intestinal tissues is a LEE-dependent phenotype, which is congruent with decreased LEE expression by galacturonic acid. To further dissect the contribution of pectin degradation by *Bt* in *C. rodentium* infection of mouse, mice in which microbiota were depleted by antibiotic treatment were monocolonized with *Bt* or *Bt* ΔPUL75 (which does not...
\textbf{Fig. 5 | Regulation of the T3SS by ExuR in vivo is independent of its role in regulating galacturonic acid metabolism.} \textbf{a.} Model depicting a simplified schematic of the Ashwell pathway for galacturonic and glucuronic acid utilization. \textbf{b.} Bacterial c.f.u. in stools of C3H/HeJ mice infected with WT (n = 16) or \(\Delta\uxaC\) (n = 16). \(n\), number of biological replicates; results are representative of two independent experiments. Shown are mean and \(P\) values (two-sided, two-way ANOVA). \textbf{c.} Groups of C3H/HeJ mice were intragastrically inoculated with a 1:1 ratio of the WT (CarbR) (pWSK29; carbenicillin resistant), WT (KmR) (pWSK129; kanamycin resistant) (n = 8) or WT and \(\Delta\uxaC\) (n = 12) \textit{C. rodentium} strains. Samples were collected 2 d post-infection. Competitive indices were calculated using the relative abundance of each strain in the caecal content, corrected by the ratio in the inoculum. \(n\), number of biological replicates; results are representative of two independent experiments. Shown are mean and \(P\) values (two-sided Mann–Whitney test). \textbf{d.} \textit{RT–qPCR} of the LEE-encoded gene \(espA\) from the caecal content of mice infected with WT (n = 12) or \(\Delta\uxaC\) (n = 12) collected 2 d post-infection. \(n\), number of biological replicates; results are representative of three independent experiments. Shown are mean and \(P\) values (two-sided, two-Way Mann–Whitney test). \textbf{e.} Bacterial c.f.u. in stools of C3H/HeJ mice infected with WT (n = 12) or \(\Delta\uxaC\) (n = 12), \(\Delta\exuR\) (n = 12) or \(\Delta\uxaC\Delta\exuR\) (\(\Delta\Delta\)) (n = 12) \textit{C. rodentium} at 2, 4, 6 and 8 d post-infection. \(n\), number of biological replicates; results are representative of three independent experiments. Shown are mean and \(P\) values (two-sided, two-way Kruskal–Wallis test). \textbf{f.} Bacterial c.f.u. in caecal tissue of C3H/HeJ mice infected with WT (n = 16) or \(\Delta\uxaC\) (n = 16), \(\Delta\exuR\) (n = 16), \(\Delta\uxaC\Delta\exuR\) (\(\Delta\Delta\)) (n = 16) \textit{C. rodentium} 8 d post-infection (peak of disease) (n, number of biological replicates; results are representative of three independent experiments). Shown are mean and \(P\) values for two-sided two-way Kruskal–Wallis statistical analysis. \textbf{g.} Survival curves of C3H/HeJ mice infected with WT (n = 8) or \(\Delta\uxaC\) (n = 8), \(\Delta\exuR\) (n = 8) or \(\Delta\uxaC\Delta\exuR\) (\(\Delta\Delta\)) (n = 8) \textit{C. rodentium}. Two-sided log-rank (Mantel–Cox) test. \textbf{h.} Competitive indices of C3H/HeJ mice, untreated or treated with DSS infected with equal mixtures of the indicated strains in the caecal content. Two days after infection, samples were collected for analysis. Data are mean ± s.e.m.; \(n = 8\) per group; two-sided Mann–Whitney test.

degrade pectin\textsuperscript{43}, treated with or without pectin and infected with \textit{C. rodentium}. There were no differences in \textit{C. rodentium} loads in caecum content of these animals (Fig. 6g). However, in mice treated with pectin, \textit{C. rodentium} colonization of caecum tissues was decreased only in those mice that were colonized with \textit{Bt} proficient in pectin degradation (Fig. 6h); this phenotype was dependent on decreased expression of the LEE (Fig. 6i). Consequently, pectin degradation to galacturonic acid by \textit{Bt} in the gastrointestinal tract

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\(\Delta\uxaC\)

\(\Delta\uxaR\)

\(\Delta\Delta\)

\(\Delta\exuR\)

\(\Delta\uxaC\Delta\exuR\)
decreases LEE gene expression and pathogen adherence to intestinal tissues.

Consequently, diet-derived galacturonic acid released by the microbiota in the lumen confers an initial growth advantage to C. rodentium during early intestinal colonization. This is achieved by utilization of galacturonic acid as a carbon source and the decreased energetic burden of LEE gene expression. However, as infection progresses and carbon sources are depleted, LEE expression is activated, leading to tissue attachment, lesion formation and inflammation. This switch is coordinated by ExuR sensing of galacturonic acid.

**Discussion**

The nutritional requirements that support colonization by enteric pathogens are not fully characterized. Competition for available nutrients imposes a barrier against colonization, a concept termed colonization resistance. Pathogens utilize distinct metabolic strategies from the resident microbiota to avoid competition for similar nutrients. Because of its very small infectious dose (estimated at 50 c.f.u.), EHEC has evolved to utilize different nutrient sources than closely related commensals to avoid direct competition. Notably, utilization of sugar acids is required for full colonization of the gut by EHEC, but not by the closely related commensal strains *E. coli* Nissle 1917 or HS. Attaching and effacing pathogens such as EHEC and *C. rodentium* also require a T3SS to outcompete the resident microbiota and colonize the large intestine. However, expression of a T3SS also constitutes a considerable metabolic burden for these pathogens, which therefore requires precise regulation. It is noteworthy that the EHEC T3SS has a modified translocon, in which the EspA protein forms a sheath around the needle, resulting in a structure as large as a flagellum. This expensive T3SS machinery combined with the low infectious dose of EHEC requires regulation by a complex cellular regulatory network with intersecting circuits. To coordinate its metabolism and virulence strategies, EHEC utilizes several nutrients as both metabolites and signals. Ethanolamine, succinate and fucose are used as signals to regulate LEE and C. rodentium gene expression and to coordinate the formation of the T3SS. These studies highlight that some enteric pathogens have evolved ways of exploiting the microbiota, creating a ‘collaborative’ environment in which they thrive.

Here we show another example of microbiota–pathogen collaboration. Galacturonic acid is a carbon source made available in the digestion of dietary pectin. Another example of microbiota–pathogen collaboration is the utilization of dietary pectin. When mice are treated with an antibiotic cocktail to deplete their intestinal microbiota and subsequently inoculated with WT *Bt* at 1:1 ratio, *C. rodentium* is less competitive (Fig. 6). After monocolonization, the mice were infected with *C. rodentium* Δ*uxaA* (Fig. 6). After monocolonization, the mice were infected with *C. rodentium* Δ*uxaA* and the sugar-acid utilization genes *uxaC* and *luxA* from EHEC co-cultured with *Bt* in low-glucose DMEM supplemented with 1% GalA (n = 8) or pectin (n = 8). Competition for available nutrients is required for full colonization of the gut by EHEC, but not by the closely related commensal strains *C. rodentium* (n = 8). competition for available nutrients is required for full colonization of the gut by EHEC, but not by the closely related commensal strains *C. rodentium* (n = 8). Competition for available nutrients is required for full colonization of the gut by EHEC, but not by the closely related commensal strains *C. rodentium* (n = 8).
by EHEC and C. rodentium in the gut as a carbon source, aiding these pathogens’ initial expansion 10 (Fig. 5). Galacturonic acid is sensed through ExuR, which in the absence of this sugar acid, presumably as infection progresses, acts as a transcriptional activator of the LEE (Figs. 1 and 2). Importantly, this ExuR–galacturonic acid regulation has a key role in the establishment and progression of C. rodentium mouse infection (Figs. 3–5). In this scenario, the pathogen’s ability to gauge the concentration of galacturonic acid and its ability to utilize it both as a nutrient and as a signal have key roles in its adaptation to the gastrointestinal environment and its ability to outcompete the microbiota to find a suitable colonization niche.

The relationships among host, microbiota and enteric pathogens are complex and multi-layered. They involve a high level of integration between metabolic circuits and virulence gene expression. This work is a step towards defining the molecular mechanisms that govern how commensal species influence virulence of an intestinal pathogen. It has fundamental implications for how differential compositions of microbiota may affect disease outcome and susceptibility to pathogens.

Methods

Strains, plasmids and culture conditions. Strains and plasmids used in this study are listed in Table S1. WT EHEC O157:H7 strain 86–244, C. rodentium (DBS100)22 and isogenic mutants were routinely grown at 37 °C in Luria–Bertani (LB) medium overnight. Bt VPI-5482 was routinely grown anaerobically overnight at 37 °C in TYG medium. To express T3SS, low-glucose (1 g l−1) DMEM was used at 37 °C as these conditions have been shown to induce T3SS under microaerobic conditions2,23. Anaerobic growth was carried out using either GasPak EZ anaerobe (Becton Dickinson) or Bactron EZ anaerobic chamber (Sheldon Manufacturing). For co-culture experiments with Bt and EHEC, the strains were grown overnight as described above and then grown anaerobically in low-glucose DMEM with a 10:1 ratio of Bacteroides/EHEC supplemented with 1% galacturonic acid or citrus-peel pectin (Sigma, catalogue (cat.) no. 9000-69-5).

Recombinant DNA techniques. All primers used for mutant and plasmid construction are shown in Supplementary Table 2.

Mice. Mice were housed under specific pathogen-free conditions and maintained on a 12 h:12 h light:dark cycle with access to ad libitum access to food (5053 Rodent diet 20, Picolab) and water, unless otherwise noted. We used 8–12-week-old female CH1/HeJ or C57BL/6j mice (Jackson Laboratories). We used female mice to facilitate randomized mixing between the experimental groups before every experiment. Researchers were not blind to the experimental groups and sample size was chosen empirically. Mice were used within 6 weeks of the C. rodentium infection studies. All experiments were performed using protocols approved by University of Texas Southwestern’s Institutional Animal Care and Use Committee (IACUC).

Epithelial cells. HeLa cells were obtained from ATCC and stored in liquid nitrogen until used. HeLa cells were not independently authenticated by our laboratory. They were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin–gentamicin antibiotic mix at 37 °C at 5% CO2. They were screened for Mycoplasma by PCR with primers designed to detect Mycoplasma 16S RNA.

Isogenic mutant construction. Construction of EHEC ΔeaeA and ΔusuC was performed using lambda-red mutagenesis19. The primers used to construct mutants are shown in Supplementary Table 2. In brief, a PCR product was generated using primers containing homologous regions to sequences flanking the eaeA and usuC genes to amplify a kanamycin-resistance gene from pKD4. EHEC 86–24 and C. rodentium DBS100 cells harbouring pKD46 were electroporated using the PCR product and colonies were selected from kanamycin LB plates. Nonpolar mutants were generated using Flp recombinases encoded in pCP20 plasmid to cleave off the kanamycin cassette. Complementation experiments were conducted using PCR products flanked with restriction enzymes; the PCR products were generated from EHEC 86–24 genomic DNA, amplifying exuR, and were cloned into pACYC184.

RNA extraction and RT–qPCR. Primers used in RT–qPCR and cloning are listed in Supplementary Table 2. RNA from three biological replicates was extracted using a Ribopure kit (Ambion) according to the manufacturer’s instructions. For in vitro experiments, cultures were grown to mid-log (optical density at 600 nm (OD600) of 0.6) in low-glucose DMEM under microaerobic conditions. RNA was extracted using the Ribopure bacterial isolation kit (Ambion) according to the manufacturer’s protocols. For in vivo experiments, caecal contents were collected from infected mice on day 8 after C. rodentium infection, flash frozen in liquid nitrogen and stored at −80 °C. The frozen contents were homogenized for two cycles of 45 s in a bead beater (BioSpec) and RNA was isolated using the RNeasy PowerMicrobeone kit (QIAGEN). The primers used for RT–qPCR were validated for amplification efficiency and template specificity. RT–qPCR was performed as follows. In brief, 2 µg of diluted extracted RNA was converted to cDNA with addition of Superscript (Thermo Fisher Scientific), random primers, dTTP and dNTPs. Validated primers (Supplementary Table 2) and SYBR Green (Thermo Fisher Scientific) were added to the cDNA and the mix was run in QuantiStudio 6 flex (Applied Biosystems). Data were collected using QuantiStudio Real-Time PCR Software v1.3, normalized to endogenous rp03 and rp02 levels, and analysed using the comparative critical threshold (Ct) method. The Student’s paired t-test was used to determine statistical significance. A P value of <0.05 was considered significant.

Fluorescent actin staining assay. Fluorescein actin staining assays were performed as previously described7. In brief, HeLa cells were grown to 80% confluence. Wells were washed with PBS and replaced with low-glucose DMEM supplemented with 10% fetal bovine serum. Bacterial cultures were grown aerobically overnight as described above at 37 °C. Overnight bacterial cultures were diluted 1:100 in low-glucose DMEM and grown for 4 h. This culture was used to infect confluent monolayers of HeLa cells for 3 h at 37 °C and 5% CO2. After 3 h of infection, the coverslips were washed, fixed, permeabilized and then treated with FITC-labelled phallobin to visualize actin accumulation. The coverslips were mounted on slides and visualized with a Zeiss Axiosvert microscope. The number of bacteria attached per HeLa cell was quantified. Replicate coverslips from multiple experiments were quantified and statistical analyses were performed.

Protein purification and electrophoretic mobility shift assay. ExuR was cloned into the Nde I and BamH I sites of pASK-IBA32 by Gibson cloning, Table S2, to create an N-terminal His-tagged construct. This was transformed into BL21 cells. His-tagged ExuR was purified using the AKTA START system with pre-packed nickel columns according to manufacturer’s instructions. For electrophoretic mobility shift assay (EMSA), DNA probes were prepared by PCR from genomic templates. Probes were purified by DNA electrophoresis and agarose gel extraction using a QIAquick Gel Extraction Kit from QIAGEN per the manufacturer’s instructions. The amplicons were labelled with [32P]–γ-ATP using T4 PNK (NEB). Labelled probes were further purified by Qiagen PCR purification kit. EMSA reactions were performed in 50 mM NaPO4 pH 7.5, 100 mM NaCl, 1.5 mM DTT, 100 µg ml−1 BSA, and 250 µg ml−1 DNAse to produce the indicated DNA:protein concentrations. Binding was resolved on 5% polyacrylamide gels in Tris–borate–EDTA. Gels were dried onto filter paper and exposed to phosphoimager screens and assessed on a GE Typhoon scanner.

Western blotting of secreted and lysate fractions. Secreted proteins were prepared as previously described16. Briefly, cultures were grown microaerobically in low-glucose DMEM at 37 °C and collected at an OD600 of 0.6. Total secreted proteins from culture supernatants were separated from bacterial cells using centrifugation and filtration. The proteins were separated by SDS–PAGE and analysed by immunoblotting with rabbit polyclonal antiserum to EspA and EspB and visualized with enhanced chemiluminescence (Bio-Rad). Whole-cell lysates were prepared from strains grown microaerobically in low-glucose DMEM and collected at a density of OD600 (during growth phase). Cells were collected by centrifugation and then lysed with urea lysis buffer (100 mM NaH2PO4, 10 mM Tris, 8 µM urea and pH 6.3). Samples were probed using antisera against EspA, EspB and Tir.

In vitro competition experiments. Bacteria were grown anaerobically for 48 h in minimal medium containing 100 mM KH2PO4 (pH 7.2), 15 mM NaCl, 8.5 mM (NH4)2SO4, 4 mM l-cysteine and 1.9 µM haemin in 200 µl of 1-histidine, 100 mM MgCl2, 1.4 µM FeSO4, 7H2O, 50 mM CaCl2, 1 µM l-vitamin K3 and 5 mM l-vitamin B12. Mucin and pectin were added to the minimal medium to a final concentration of 1%. Media were filter sterilized using a Millipore Express filter unit (0.22 µm pore diameter). The culture was centrifuged at 5,900 × g, the supernatant was collected and filtered through a 0.22 µm filter. Supernatants were inoculated with a 1:1 ratio of C. rodentium and the competitive index was determined 24 h after inoculation by plating on selective agar plates.

C. rodentium infection. C. rodentium was grown overnight in LB broth supplemented with nalidixic acid (NaLA,30 µg ml−1) with shaking at 37 °C. Mice were infected by oral gavage with 100 µl of PBS containing approximately 1 × 108 c.f.u. of C. rodentium. To determine the bacterial numbers in the faeces, faecal...
pellets were collected from individual mice, weighed, and homogenized in cold PBS and plated at serial dilutions onto LB agar containing 30 μg mL−1 NaA, and the number of c.f.u. was determined after overnight incubation at 37 °C. Mice were killed at various time points after infection. The caecum content and tissue were collected to determine the c.f.u. and for RT-qPCR analysis. Caecal patches were fixed in 10% formalin and then processed for haematoxylin and eosin staining. In competitive infections, a 1:1 ratio of two C. rodentium strains were administered via orogastric gavage at a combined final concentration of 1 × 107 c.f.u. per mouse. Caecal contents were collected 2 d after infection and placed into sterile PBS and serially diluted on selective agar plates to determine c.f.u. per g of each strain. The competitive indices were calculated by dividing the c.f.u. per g of WT C. rodentium recovered over the c.f.u. per g of mutant strain recovered, and later normalized to the same ratio in the inoculum. For the pectin-diet experiments we used 8–12-week-old female C57Bl/6 mice (Jackson Laboratories). From arrival, the mice were switched to a control diet TD.94096 (200.0 g kg−1 casein, 3.0 g kg−1 dl-methionine, 494.787 g kg−1 sucrose, 150 g kg−1 corn starch, 50 g kg−1 corn oil, 50 g kg−1 cellulose, 35 g kg−1 Mineral Mix AIN-76, 15 g kg−1 Vitamin Mix AIN-76A, 2.2 g kg−1 choline bitartrate, 0.003 g kg−1 vitamin K, MSB complex and 0.01 g kg−1 ethoxyquin, antioxidant) or a 5% citrus-peel pectin (cat. no. 9000-69-5) diet (17. Rodionov, D. A., Mironov, A. A., Rakhmaninova, A. B. & Gelfand, M. S. Genetic analysis of enterococcal extracytoplasmic function genes: evidence for ExuR and ExuX genes: evidence for ExuR and ExuX monomer repressors integration in pathogen–microbiota–host interactions. Mol. Gen. Genet. 199, 507–511 (1985). 18. Njoroge, J. W., Nguyen, Y., Curtis, M. M., Moreira, C. G. & Sperandio, V. Redox, nutrient, and fatty acid metabolism intersect with bacterial virulence in the gut. Proc. Natl Acad. Sci. USA 115, E10712–E10719 (2018). 19. Tutukina, M. N., Potapova, A. V., Cole, J. A. & Ozoline, O. N. Control of hexuronic metabolism in Escherichia coli by the two interdependent regulators, ExuR and ExuX deregulation by heterodimer formation. Microbiology 162, 1220–1229 (2016). 20. Mellies, J. L., Elliott, S. J., Sperandio, V., Donnenberg, M. S. & Kaper, J. B. The Per regulon of enteropathogenic Escherichia coli: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Let). Mol. Microbiol. 33, 296–306 (1999). 21. Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S. & Kaper, J. B. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic Escherichia coli. Proc. Natl Acad. Sci. USA 96, 15196–15201 (1999). 22. Njoroge, J. W., Nguyen, Y., Curtis, M. M., Moreira, C. G. & Sperandio, V. Virulence meets metabolism: Cra and KdpE gene regulation in enterohemorrhagic Escherichia coli. mbio 3, e00280–00212 (2012). 23. Borenstein, D., Nambiar, P. R., Groff, E. R., Fox, J. G. & Schauer, D. B. Development of fatal colitis in FVB mice infected with Citrobacter rodentium. Infect. Immun. 75, 3271–3281 (2007). 24. Winter, S. E. et al. Host-derived nitrate boosts growth of E. coli in the inflamed gut. Science 339, 708–711 (2013). 25. Carlson-Banning, K. M. & Sperandio, V. Catabolite and oxygen regulation of enterohemorrhagic Escherichia coli virulence. mBio 7, e01852–16 (2016). 26. Mundy, R., MacDonald, T. T., Dougan, G., Frankel, G. & Wiles, S. Citrobacter rodentium of mice and man. Cell Microbiol. 17, 1697–1706 (2015). 27. Mohnen, D. Pectin structure and biosynthesis. Carr. Optins. Plant Biol. 11, 266–277 (2008). 28. Luis, A. S. et al. Dietary pectic glycans are degraded by coordinated enzyme pathways in human colonic Bacteroides. Nat. Microbiol. 3, 210–219 (2018). 29. Ndiel, D. et al. Complex pectin metabolism by gut bacteria reveals novel catalytic functions. Nature 544, 65–70 (2017). 30. Curtis, M. M. et al. The gut commensal Bacteroides thetaiotaomicron exacerbates enteric infection through modification of the metabolic landscape. Cell Host Microbe 16, 759–769 (2014). 31. Umar, S., Morris, A. P., Kearton, E. & Sellin, J. H. Dietary pectin and calcium inhibit colonic proliferation in vivo by differing mechanisms. Cell Prolif. 56, 361–375 (2003). 32. Kuss, S. K. et al. Intestinal microbiota promote enteric virus replication and systemic pathogenesis. Science 334, 249–252 (2011). 33. Sassone-Corsi, M. & Raffatelli, M. No vacancy: how beneficial microbes cooperate with immunity to provide colonisation resistance to pathogens. J. Immunol. 194, 4081–4087 (2015). 34. Cameron, E. A. & Sperandio, V. Frenemies: signaling and nutritional integration in pathogen–microbiota–host interactions. Cell Host Microbe 18, 273–284 (2015). 35. Pacheco, A. R. & Sperandio, V. Enteric pathogens exploit the microbiota-generated nutritional environment of the gut. Microbiol. Spectr. 3, MBP-0001-2014 (2014). 36. 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. 86, 132–137 (1954).
37. Pacheco, A. R. et al. Fucose sensing regulates bacterial intestinal colonization. *Nature* **492**, 113–117 (2012).
38. Knutton, S. et al. A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO J.* **17**, 2166–2176 (1998).
39. Kendall, M. M., Gruber, C. C., Parker, C. T. & Sperandio, V. Ethanolamine controls expression of genes encoding components involved in interkingdom signaling and virulence in enterohemorrhagic *Escherichia coli* O157:H7. *mBio* **3**, e00250–12 (2012).
40. Gonyar, L. A. & Kendall, M. M. Ethanolamine and choline promote expression of putative and characterized fimbriae in enterohemorrhagic *Escherichia coli* O157:H7. *Infect. Immun.* **82**, 193–201 (2014).
41. Porter, N. T., Lu, A. S. & Martens, E. C. *Bacteroides thetaiotaomicron*. *Trends Microbiol.* **26**, 966–967 (2018).
42. Griffin, P. M. et al. Illnesses associated with *Escherichia coli* O157:H7 infections. A broad clinical spectrum. *Ann. Intern. Med.* **109**, 705–712 (1988).
43. Barthold, S. W., Coleman, G. L., Bhatt, P. N., Osbaldiston, G. W. & Jonas, A. M. The etiology of transmissible murine colonic hyperplasia. *Lab. Anim. Sci.* **26**, 889–894 (1976).
44. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl Acad. Sci. USA* **97**, 6640–6645 (2000).
45. Knutton, S., Baldwin, T., Williams, P. H. & McNeish, A. S. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. *Infect. Immun.* **57**, 1290–1298 (1989).

**Acknowledgements**

This study was supported by the National Institutes of Health grants AI053067, AI05135, AI077613 and AI114511 to V.S. A.G.J. was supported through National Institutes of Health Training Grant 5 T32 AI7520.

**Author contributions**

A.G.J. conceived the studies, performed experiments and data analysis and wrote the paper. M.E. performed histological analysis and performed some mouse experiments. W.A. advised on experiments with *Bt* and pectin degradation. V.S. supervised all experiments, analysed data and wrote the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41564-019-0641-0. Supplementary information is available for this paper at https://doi.org/10.1038/s41564-019-0641-0.

**Correspondence and requests for materials** should be addressed to VS.

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Extended Data Fig. 1 | ExuR regulates genes encoding enzymes necessary for the catabolism of galacturonic-acid. a, Schematic representation of ExuR repression of the galacturonic-acid utilization genes. b, qRT-PCR of the galacturonic utilization genes in WT (n = 3) and ΔexuR (n = 3) EHEC (n, number of biological replicates; results are representative of two independent experiments). The mean and P value for two-sided Mann-Whitney statistical analysis are shown. c, Competition EMSA of ExuR binding to the ler promoter in the absence and presence of ler cold probe. Results are representative of two independent experiments with similar results. d, qRT-PCR of the LEE espA gene in EPEC in the presence of vehicle (n = 6) or galacturonic-acid (GalA) (n = 6) (n, number of biological replicates; results are representative of two independent experiments). The mean and P value for two-sided Mann-Whitney statistical analysis are shown.
Extended Data Fig. 2 | UxaC and galacturonic acid promotion of EHEC’s growth. **a**, Growth curves of WT (n = 2) and ΔuxaC (n = 2) EHEC in M9 minimal medium with 5mM galacturonic-acid as a sole carbon source (n, number of biological replicates; results are representative of one independent experiments). The mean ± SD and P value for two-sided two-way ANOVA statistical analysis are shown. **b**, Growth curves of WT (n = 4) and ΔuxaC (n = 4) EHEC in low-glucose DMEM (n, number of biological replicates; results are representative of one independent experiments). The mean and P value for two-sided one-way ANOVA statistical analysis are shown. **c**, Growth of WT EHEC in low-glucose DMEM supplemented with different concentrations of galacturonic acid.
Extended Data Fig. 3 | ExuR also activates the LEE in *C. rodentium*. **a**, Growth curve of WT (*n* = 3) and ΔexuR (*n* = 3) *C. rodentium* strains grown in low-glucose DMEM under microaerobic conditions (*n*, number of biological replicates; results are representative of one independent experiments). The mean ± SD value for two-sided one-way ANOVA statistical analysis are shown. **b**, RT-qPCR of the LEE-encoded genes in escC, escV, tir, and espA in WT (*n* = 9) and ΔexuR (*n* = 9) *C. rodentium* (*n*, number of biological replicates; results are representative of three independent experiments). The mean and *P* value for two-sided two-way ANOVA statistical analysis are shown. **c**, Western blot for secreted EspB in WT and ΔexuR *C. rodentium*. Representative blots from three independent experiments. **d**, Growth curves of *C. rodentium* with glucose (Glu) (*n* = 3), galacturonic acid (GalA) (*n* = 3) or glucuronic acid (GlcA) (*n* = 3) as sole carbon sources (*n*, number of biological replicates; results are representative of three independent experiments). The mean ± SD value for two-sided one-way ANOVA statistical analysis are shown.
Extended Data Fig. 4 | Galacturonic-acid acts through ExuR to decrease LEE gene expression. Western blot of supernatants from EHEC WT, ΔexuR, ΔuxuR and ΔexuRuxuR (ΔΔ) grown in the presence of glucose (Glu), galacturonic-acid (GalA), or glucuronic acid (GlcA) in DMEM probed with anti-EspA antiserum. BSA is used as a loading control. Representative blots from three independent experiments.
Extended Data Fig. 5 | Mice infected with ΔuxaC C. rodentium have only a mild increase in inflammation. C3H/HeJ mice under non-infected conditions as well as at post-infection day 8 with WT or the ΔexuR, ΔuxaC and ΔexuRΔuxaC mutants. a, Haematoxylin-eosin-stained cecal patch tissues of C3H/HeJ mice. Representative images from two independent experiments, scale bars = 100 μm. b, Blinded histopathology scores of non-infected mice or infected with WT (n = 4) or the ΔexuR (n = 4), ΔuxaC (n = 4), and ΔexuRΔuxaC (n = 4) C. rodentium (n, number of biological replicates; results are representative of two independent experiments). The mean and P value for two-sided one-way ANOVA statistical analysis.
Extended Data Fig. 6 | Pectin engenders inflammation in C3H/HeJ mice. C3H/HeJ mice were treated with 200µL of 2% pectin or PBS. a, Blinded histopathology scores of non-infected mice treated with 200µL of 2% pectin (n = 4) or PBS (n = 4) (n, number of biological replicates; results are representative of two independent experiments). The mean and P value for two-sided one-way ANOVA statistical analysis. b-c, RT-qPCR determined the expression of Nos2 and IL22 genes in the cecal tissue of mice treated with 200µL of 2% pectin (n = 4) or PBS (n = 4) (n, number of biological replicates; results are representative of two independent experiments). The mean and P value for two-sided Mann-Whitney statistical analysis are shown. GAPDH was used to normalize gene expression.
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Software and code

Policy information about availability of computer code

Data collection

See method section for RNASeq analyses. The raw data generated was analyzed using DNASTAR Lasergene software. All experiments were normalized by reads assigned per kilobase of target per million mapped reads (RPKM). RNAseq data can be accessed at European Nucleotide Archive accession number: PRJEB30676.

Data analysis

See method section for RNASeq analyses. The raw data generated was analyzed using DNASTAR Lasergene software. All experiments were normalized by reads assigned per kilobase of target per million mapped reads (RPKM). RNAseq data can be accessed at European Nucleotide Archive accession number: PRJEB30676.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size  Sample sizes were determined empirically and according to extensive literature on the C. rodentium infection models, and appropriate statistical analyses were performed. Sample sizes for qRT-PCR were performed by us and other always using at least 3 biological replicas with 3 technical replicates of each. The rpoA gene was always used as an internal control, for mammalian qRT-PCR the internal control was GAPDH.

Data exclusions  N/A

Replication  Animal infections were repeated in three independent cohorts. In vitro data used at least 3 biological samples and 3 technical replicates in at least three independent experiments.

Randomization  All mice were randomized to avoid cage effects.

Blinding  Pathology scores were randomized and scored blindly

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems  n/a

| Involved in the study |
|-----------------------|
| Antibodies |
| ☒ Eukaryotic cell lines |
| ☐ Palaeontology |
| ☐ Animals and other organisms |
| ☐ Human research participants |
| ☐ Clinical data |

Methods  n/a

| Involved in the study |
|-----------------------|
| ☐ ChIP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |

Antibodies

| Antibodies used |
|----------------|
| Anti-EspA, EspB and Tir antibodies were polyclonal antibodies generated in rabbits from recombinantly purified proteins. They were IgA purified, absorbed against espA, espB and tir mutants respectively, and ran in controls westerns against these mutants to ensure no cross-detection. These are not commercially available. |

| Validation |
|------------|
| Isogenic espA, espB and tir mutants were used to validate the specificity of these antibodies. Results using these antisera were previously published (Cell Host and Microbe 16:759-769; mBio. 14;5(1). pii: e01025-13; mBio 11;5(6). pii: e02165-14; mBio. Volume 7 Issue 3 e00826-16; mBio Nov 22;7(6). pii: e01852-16; Cell Host Microbe. 9;23(5):607-617) |

Eukaryotic cell lines

| Policy information about cell lines |
|-----------------------------------|
| ☐ HeLa cells ATCC |

| Authentication |
|----------------|
| Previously published and extensively used in the laboratory as well as throughout the department. No further authentication was used. |

| Mycoplasma contamination |
|--------------------------|
| They were screened by PCR by micoplasma infection. They all tested negative. |

| Commonly misidentified lines (See ICLAC register) |
|--------------------------------------------------|
| not used |


## Animals and other organisms

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| Category                     | Description                                                                 |
|------------------------------|-----------------------------------------------------------------------------|
| Laboratory animals           | Species is mice. Strain is C3H/HeJ. Sex was female. Age 8-12 week old.       |
| Wild animals                 | not used                                                                    |
| Field-collected samples      | not used                                                                    |
| Ethics oversight             | All experiments were performed using protocols approved by UT Southwestern’s institutional animal care and use committee (IACUC). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.