Dietary L-arabinose-induced gut dysbiosis exacerbates bacterial infection

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Abstract

Background

Gut microbiota is essential for providing colonization resistance against pathogens. Dietary sugars markedly shift the composition of the intestinal microbiota and alter host susceptibility to enteric infections. However, the role of dietary sugars in intestinal pathophysiology and colitis pathogenesis remains controversial.

Results

We demonstrate the effect of L-arabinose on bacterial infection by using Salmonella enterica serovar Typhimurium (S. Tm). L-arabinose triggers severe inflammation in the gut and aggravates systemic infection of S. Tm in conventional mice. In addition, L-arabinose represses the expression of Salmonella Pathogenicity Island 1 (SPI-1) genes by negatively regulating the activity of the cyclic 3’ 5’-AMP (cAMP)-cAMP receptor protein (CRP) complex. The cAMP-CRP complex activates yifA to maintain the stability of HilD. In a streptomycin-pretreated mouse model, L-arabinose supplementation promotes S. Tm initial bloom and is unable to alter the disease progression of Salmonella infection. However, in the presence of microbiota, L-arabinose induces a dramatic expansion of Enterobacteriaceae, thereby decreasing the microbiota diversity and causing more severe systemic infections.

Conclusions

Our work reveals that a high intake of dietary L-arabinose disrupts gut homeostasis in response to enteric infections, which offers new perspectives for dietary strategies and supplementation for diabetics.

Introduction

The scavenging of nutrients is the first step for pathogens to occupy and proliferate in the gut, and microbiota composition, host genetic conditions or antibiotic treatment influences the course of enteric infections (1). The accessibility of dietary sugars by pathogenic bacteria is crucial for successful infection of the host (2). Salmonella enterica serovar Typhimurium (S. Tm) is a facultative intracellular pathogen that produces both localized gastroenteritis and disseminated systemic disease in humans and animals (3). Type III secretion system 1 (T3SS-1) is a key virulence factor, which is generally employed by S. Tm for epithelial internalization and subsequent inflammation. T3SS-1 is encoded on Salmonella pathogenicity island 1 (SPI-1), a distinct region in the chromosome that defines the genus Salmonella (4). HilA is a transcriptional regulator of SPI-1 that directly activates the transcription of T3SS-1 apparatus genes. Regulatory inputs into the SPI-1 system are integrated at the level of HilD, which in turn activates hilA and initiates the expression of T3SS-1 (5). In addition, transcription of hilD is controlled by several host and microbiota-derived signals, such as propionate (6), chenodeoxycholic acid (7), and oxygen (8).
L-arabinose, an abundant monosaccharide in plants, has been proven to selectively inhibit intestinal sucrase activity in an uncompetitive manner and suppress diet-induced obesity in humans (9). Although L-arabinose is poorly absorbed in the human gut, many gut bacteria use it as a source of carbon and energy. L-arabinose uptake in \textit{S. Tm} is mediated by AraE permease (10). The expression of the \textit{araBAD} operon encoding enzymes for metabolizing L-arabinose to D-xylulose-5-phosphate for the pentose phosphate pathway is induced by intracellular L-arabinose (11). It has been illustrated that L-arabinose metabolism inhibits \textit{S. Tm} biofilm formation in response to intracellular c-di-GMP levels (12). HilD is likely to be repressed by L-arabinose at the post-transcriptional level, which is independent of L-arabinose metabolism (13). However, the underlying mechanism of SPI-1 repression exerted by L-arabinose remains elusive.

Simple sugar metabolism plays a crucial role in the ecophysiology of the human gut microbiota, which overrides host genetic effects (14, 15). For example, L-arabinose suppresses diet-induced obesity in mice in the presence of sucrose and serves as a modulator to preserve host gut-microbiota homeostasis (16). Notably, L-arabinose is utilized by both pathogens and commensals to establish or maintain host colonization, and such competition modifies the within-host environment in inflamed guts and shapes different infection outcomes (17–19). However, the beneficial effects of L-arabinose have mostly been investigated on uninfected humans or animals (20, 21). The role of L-arabinose in infected or inflamed guts remains largely unknown. L-arabinose seems to modulate enteric infections, serving as both an environmental signal and a nutrient in the host intestine. Since L-arabinose is a dietary supplement for diabetic patients, who are usually associated with enteric infections and microbiota dysbiosis, it is urgent to clarify the role of L-arabinose in enteric infections.

Streptomycin-pretreated mice are mostly used to study colitis caused by \textit{Salmonella} infections (22). However, disruption of the commensal community in this model is unable to investigate the relationship between pathogens and microbiota during enteric infections, and such models are not fully reminiscent of typhoidal salmonellosis in humans (23–25). In contrast, gastrointestinal infection with \textit{S. Tm} leads to spread via gut-associated lymphoid tissues to systemic sites in conventional mice, and symptoms are similar to those of typhoidal salmonellosis in humans. In this study, we showed that L-arabinose exacerbated intestinal inflammation and accelerated \textit{Salmonella} systemic infections. Particularly, L-arabinose-treated mice resulted in a loss of gut microbiome diversity and overgrowth of \textit{Enterobacteriaceae} compared with untreated mice upon \textit{Salmonella} infection. L-arabinose also reduced the survival rate of \textit{Salmonella}-infected hyperglycemic mice. Taken together, our findings elucidate the role of L-arabinose in bacterial infections and provide new perspectives for understanding the metabolic interactions that connect with virulence gene regulation in enteric infections.

Results

\textbf{L-arabinose exacerbates \textit{Salmonella} infection in a mouse typhoid model.}
L-arabinose has been proven to be a remarkable candidate as a functional food for type 2 diabetes mellitus (20), but diabetic patients are in an immunocompromised state and are usually associated with various types of infections including colitis (26). To understand the effect of dietary L-arabinose on bacterial colitis, we fed mice with 300 mM L-arabinose in drinking water following S. Tm administration by oral gavage. This dose of L-arabinose is clinically relevant because human clinical trials show that 4% L-arabinose addition in sucrose beverages reduces postprandial glucose, insulin and augments the postprandial increase in the GLP-1 response (9). To our surprise, the survival rate of L-arabinose-treated mice was significantly decreased compared with that of control mice (Fig. 1A). Notably, mice body weight loss was also higher in the L-arabinose-treated group (Fig. 1B). S. Tm is able to exploit L-arabinose as a nutrient, so the results of bacterial burden in the liver and spleen showed that the wild type strain (WT) outcompeted the ΔaraA strain during L-arabinose administration (Figure S1A). The AraE permease is essential for transport of L-arabinose, but a high extracellular L-arabinose concentration (> 1%) permits transport by AraE-independent routes (13). The competitive index (CI) value of WT/ΔaraE was nearly 1, indicating that the L-arabinose concentration in the intestinal lumen was higher than 1% (Figure S1B).

In agreement with the decreased survival rate, an intake of L-arabinose plays a role in exacerbating colitis pathogenesis in mice. Histopathological analysis showed increased inflammatory changes and architectural distortion in the colon of L-arabinose-treated mice (Fig. 1C). Increased cecal bacteria (Figure S1C) and polymorphonuclear neutrophil (PMN) infiltration (Figure S1D) supported the more severe colitis phenotype following L-arabinose treatment. In addition, the colon lengths of L-arabinose-treated mice were shorter than those of untreated controls (Fig. 1D). Elevated concentrations of lipocalin-2 (LCN2), a marker of gut inflammation, verified that L-arabinose elicited a serious immune response (Fig. 1E).

To further confirm the possibility of L-arabinose driving exacerbated intestinal infection, we examined the expression of host key genes involved in inflammation, epithelial repair, and innate defense. The expression of proinflammatory cytokines including Il-6 and Tnf-a was higher in the colons of L-arabinose-treated mice on day 4 post-infection (Figure S1E). Consistently, the transcriptional levels of the host antibacterial genes Reg3b and Reg3g were increased by L-arabinose treatment (Figure S1E). Given the above observations, we concluded that L-arabinose exacerbated intestinal inflammation in bacterial-induced colitis.

Increased gut inflammation is associated with an elevated intestinal permeability and impaired tight-junction integrity (27, 28). Therefore, we began our investigation of the drivers of exacerbated enteric infection by hypothesizing that L-arabinose leads to intestinal barrier dysfunction. As expected, the FITC-dextran permeability assay showed that gut permeability increased in the L-arabinose-treated mice during Salmonella infection (Fig. 1F). The thickness of the colonic mucus layer was measured using Alcian blue/periodic acid-Schiff (AB-PAS) staining. The results revealed that mucus thickness was thinner in L-arabinose-treated mice (Fig. 1G).

Alterations in permeability allow gut-derived toxins to cross the intestinal barrier via the gut-liver axis and activate Kupffer cells in the liver, causing hepatic injury and systemic inflammation. Histological analysis
using hematoxylin and eosin (H&E) staining showed significant hepatocyte necrosis and a disordered lobule structure in the liver tissues of L-arabinose-treated mice (Figure S1F). Masson's trichrome staining also exhibited marked fatty changes with hepatic fibrosis in the L-arabinose-treated group (Figure S1G). The transcriptional level of Col1a1 (marker of hepatic fibrosis) was significantly higher in the L-arabinose-treated group (Figure S1H). L-arabinose-treated mice had more liver inflammation with higher expression levels of mRNAs encoding inflammatory cytokines and chemokines (Il-1b and Cxcl1) than control mice (Figure S1H). Collectively, these results further validated that L-arabinose promoted enteric inflammation and systemic spread, especially causing fibrosis and inflammation in the liver.

**SPI-1 repression by L-arabinose is dependent on cAMP-CRP.**

Intriguingly, L-arabinose represses DSS-induced colitis and inflammation activation (29). However, our data showed that L-arabinose exacerbated the severity of colitis induced by S. Tm infection. At the beginning of infection, S. Tm deploys T3SS-1 to overcome colonization resistance mediated by the microbiota and establish host colonization. We next ask whether L-arabinose regulates the T3SS-1 of S. Tm, further resulting in exacerbated colitis. Dietary caloric simple sugars, such as glucose, fructose, and sucrose, were also proven to aggravate DSS-induced colitis (30). To address this concern, we explored the role of various carbon sources in regulation of SPI-1 in vitro. Firstly, all tested sugars promoted S. Tm growth in LB medium (Figure S2A), indicating that these sugars can be utilized by S. Tm. We then measured the transcriptional levels of hilD and hilA by supplementation with various hexoses and pentoses in medium. The results showed that the addition of glucose, L-arabinose, or D-galactose significantly decreased the transcriptional levels of hilD (Fig. 2A) and hilA (Fig. 2B). We then determined the protein level of the SPI-1 representative effector SipB and found that L-arabinose caused a drastic decline in SipB protein level. Similarly, the protein level of SipB also decreased in the presence of glucose or D-galactose (Fig. 2C). It has been reported that virulence determinants including flagellar and T3SS-1 effectors mediate S. Tm invasion of epithelial cells (31). Therefore, we evaluated S. Tm invasion of HeLa cells by supplementation with various sugars. Congruent with the decreased expression of SPI-1 genes, bacteria treated with L-arabinose had dramatic defects in entering HeLa cells (Fig. 2D).

*Salmonella* is able to utilize metabolizable sugars to undergo glycolysis, so the pyruvic acid from glycolysis could acidify the medium. It is shown that S. Tm regulates SPI-1 expression in response to extracellular pH (32). To rule out the possibility that the regulation of SPI-1 by sugars is an indirect effect of acidification, we constructed a series of mutants which are defective in metabolizing specific sugars. Phosphofructokinase encoded by pfkA is required for glucose utilization, and we found that glucose supplementation did not alter the transcriptional levels of hilD and hilA in the deletion mutant of pfkA (Figure S2B). The galK gene encodes galactokinase for D-galactose metabolism, and the addition of D-galactose was unable to down-regulate hilD and hilA transcription in the ΔgalK strain (Figure S2C). In contrast, L-arabinose was found to repress hilD and hilA transcription in the araA deletion mutant (Figure S2D). These results suggested that repression of SPI-1 expression by L-arabinose is not dependent on arabinose metabolism, which is consistent with a previous study (13). The authors proposed that the L-arabinose-dependent repression of SPI-1 is transmitted via HilD by direct L-arabinose binding to the
We used electrophoretic mobility shift assay (EMSA) to test this hypothesis. However, the results showed that neither L-arabinose nor D-arabinose had any effect on HilD binding to hilA (Figure S2E) or hilD promoter (Figure S2F).

The phosphotransferase system (PTS) is required for the uptake of carbohydrates and mediates signal transduction and virulence in diverse pathogens (33). The PTS component EIIA\textsuperscript{Glc} (encoded by the carbohydrate repression gene, crr) mediates uptake of glucose, N-acetyl-muramic acid, and other sugars (34). The phosphorylation of EIIA\textsuperscript{Glc} could be repressed by glucose (35). When glucose is available, the phosphate group from EIIA\textsuperscript{Glc} is transformed to glucose eventually, leaving less phosphorylated EIIA\textsuperscript{Glc} to stimulate the cyclic 3’ 5’-AMP (cAMP) production. Similarly, we found that L-arabinose and D-galactose also led to dephosphorylation of EIIA\textsuperscript{Glc} (Fig. 2E), which indicated that the non-PTS sugar, L-arabinose, also decreased the intracellular cAMP of \textit{Salmonella}. As result, we speculated that extracellular L-arabinose could be sensed by cAMP-CRP, which subsequently mediates SPI-1 repression. In addition, the autoregulation circuit of the \textit{crp} plays a key role in the down-regulation of CRP by glucose (36). We found that CRP was inhibited by glucose, L-arabinose or D-galactose (Fig. 2F). As shown in Figure S2D, L-arabinose suppressed \textit{hilD} transcription in ∆araA. Thus, we constructed \textit{araA} and \textit{crp} double deletion mutant and tested the transcription of \textit{hilD} and \textit{hilA}. Strikingly, L-arabinose supplementation was unable to down-regulate SPI-1 in ∆araAΔcrp, further indicating that cAMP-CRP was required for SPI-1 repression by L-arabinose (Fig. 2G).

\textbf{cAMP-CRP activates \textit{yfiA} to regulate \textit{HilD} degradation.}

A previous study showed that the cAMP-CRP (cAMP receptor protein) complex senses different carbohydrates and regulates various cellular processes, such as bacterial biofilm formation, pathogenesis, and motility (37). cAMP-CRP acts as a metabolic sensor, which leads to the activation or repression of its target genes in response to the growth substrate. CRP is activated upon binding to intracellular cAMP generated by adenylate cyclase (CyaA). To decipher the underlying mechanism of cAMP-CRP in SPI-1 repression by L-arabinose, RNA sequencing (RNA-Seq) was performed to identify differentially expressed genes in cAMP-deficient cells. In total, the volcano plot showed that 1016 up-regulated genes and 803 down-regulated genes were found by deletion of \textit{cyaa} (Fig. 3A). The heatmap revealed that \textit{cyaa} positively regulated the expression of SPI-1 and ATP synthase genes (Fig. 3B). We verified that cellular ATP levels decreased significantly in ∆\textit{cyaa} and ∆\textit{crp}, whereas genetic complementation or exogenous cAMP restored ATP levels in mutant strains (Figure S3A). Thus, we propose that a deficiency in \textit{cyaa} shifts energy metabolism from TCA cycle to glycolysis and pentose phosphate pathway, which benefits \textit{Salmonella} to take advantage of L-arabinose for metabolism and virulence gene expression.

Both the RNA-Seq and qRT-PCR results showed that the transcriptional level of \textit{rpoH} was up-regulated in ∆\textit{cyaa} (Fig. 3A and Figure S3B). The gene of \textit{rpoH} encodes the σ\textsuperscript{32} factor for RNA polymerase, which regulates the transcription of a group of genes upon heat shock stress. Additionally, RpoH induces Lon protease, which degrades HilD (38–40). We also found that the mRNA level of \textit{lon} was elevated.
significantly in ΔcyaA, while the addition of cAMP in LB medium decreased the transcription of lon (Figure S3B). As result, the half-life of HilD in Δcrp was much shorter than that in the WT strain (Figure S3C), suggesting that cAMP-CRP post-translationally controlled HilD by affecting its stability.

As a global transcriptional regulator, cAMP-CRP functions as a dimer and binds to a symmetrical DNA sequence (consensus sequence TGTGANNNNNNNTCACA) to initiate transcription (41). To further investigate the mechanism by which cAMP-CRP exerts its control, we explored a series of promoter regions with the above binding motif. Interestingly, a putative CRP-binding site is located in the promoter region of yfiA, which encodes ribosome-associated inhibitor A (RaiA) (42). In addition, the transcriptional level of yfiA was down-regulated in cyaA deletion strain (Fig. 3A and S3B), indicating that cAMP-CRP might bind to yfiA promoter to activate its transcription. As expected, EMSA demonstrated that CRP specifically bound to yfiA promoter at a concentration of as low as 5 nM, and increasing concentration of CRP resulted in increasing CRP-promoter complex (Fig. 3C). YfiA promotes the formation of factor-bound inactive 70S ribosomes in stationary phase (43), and such factor-bound 70S ribosomes suppress protein aggregation (44). Next, a mutation in the potential binding sites of yfiA was performed to investigate the direct regulation of cAMP-CRP in vivo. We constructed a gfp translational reporter plasmid under control of the yfiA promoter (Fig. 3D). The results showed that the 5-bp mutation (P_yfiA(Mu1)) dramatically decreased the green fluorescence signals in the WT strain, and no fluorescence signal was detected in the crp deletion strain (Fig. 3E).

Western blot results showed that HilD in ΔyfiA was relatively unstable, with a half-time of 1.9 h, while the half-time of HilD in WT was 2.5 h (Fig. 3F). Additionally, the deletion of yfiA reduced Salmonella invasion of HeLa cells, which was consistent with transposon-insertion sequencing results (45). Since the overexpression of yfiA by a plasmid repressed the growth rate of Salmonella, we constructed a strain carrying the single-copy chromosomal allele of yfiA by its native promoter at the position of a pseudogene (STM14_3297) in ΔyfiA. The complementation of yfiA significantly restored the bacterial invasion ability of HeLa cells compared to ΔyfiA (Fig. 3G). Therefore, our data suggested that cAMP-CRP directly activated yfiA to control HilD degradation, thereby regulating the expression of SPI-1 genes.

L-arabinose aids the initial S. Tm expansion in streptomycin-pretreated mice.

Our above data suggested that L-arabinose repressed the expression of T3SS-1 in vitro. We wondered whether T3SS-1 is inhibited by L-arabinose in vivo. The pathogenesis of colitis caused by S. Tm in streptomycin-pretreated mice is highly dependent on the function of T3SS-1. Removal of commensal intestinal microbes by streptomycin treatment makes mice more susceptible to S. Tm infection, which shows many similarities to human Salmonella infection (46). To explore the T3SS-1 regulation by L-arabinose and to avoid microbiota interference, we employed a bioluminescent imaging (BLI) system to study the expression of T3SS-1 in a streptomycin-pretreated mouse model (47).

Firstly, we wondered whether S. Tm is accessible to exogenous L-arabinose in the murine intestinal tract in the L-arabinose-treated mouse model. Consequently, the promoter activity of araBAD was monitored in
*vivo* applying the luciferase gene cassette (*lux*) reporter system in the pACYC184 plasmid. The results showed that high-level expression by vectors containing the *araBAD* promoter was induced in LB medium (Figure S4A), indicating that this system responds to L-arabinose concentration. Accordingly, when mice were provided L-arabinose drinking water, strong bioluminescence could be detected in the intestinal tract as early as 8 h, and the signal gradually decreased during the observation period (Figure S4B and S4C). Furthermore, the competitive assay revealed that the Cl values of WT/ΔaraA were nearly 20 in the liver and spleen (Figure S4D). Taken together, these results indicated that *Salmonella* was able to utilize L-arabinose as a carbon source to replicate and cause systemic infections in the presence of L-arabinose.

To monitor the expression of T3SS-1 *in vivo*, we chose the *sicA* promoter to characterize the activity of T3SS-1 (48). We then constructed a *sicA::lux* reporter plasmid to monitor T3SS-1 in the mouse gut. Surprisingly, L-arabinose supplementation did not result in a reduced bioluminescence signal in mice (Fig. 4A). When the bioluminescence signal intensity was quantified, it was up to 5×10⁵ p/s in the L-arabinose-treated group at the time point of 8 h, which was much higher than that in the water-treated group (Fig. 4B).

The host intestinal environment is largely distinct from LB medium, and a single dose of streptomycin revealed dramatic changes in catabolism by metabolomic analysis (49). Bacteria acquire ingested food, host-synthesized gut mucus, and host circulating metabolites for catabolism, and such an environment is difficult to simulate *in vitro*. In addition, *Salmonella* liberates L-arabinose from dietary polysaccharides to promote expansion in superspreaders (50), indicating that L-arabinose is an important carbon source for *S. Tm* to survive in the gut. Therefore, we speculated that the high expression of bioluminescence signal driven by the *sicA* promoter at 8 h was due to an initial *Salmonella* bloom in the gut when providing mice with L-arabinose in water. To test this hypothesis, the constitutive promoter of the chloramphenicol acetyltransferase (*cat*) gene was fused to *lux* gene. Interestingly, we noticed that the bioluminescence signal was significantly induced in the L-arabinose-treated group at 8 h, whereas similar levels of bioluminescence signal were detected at 24 or 48 h between the two groups (Fig. 4C and 4D). Taken together, these results suggested that L-arabinose promoted early stage expansion of *Salmonella* in the gut lumen, which compensated for the repressive activity of T3SS-1 by L-arabinose in the gut.

Since the bioluminescence signal driven by the *sicA* promoter was not inhibited by L-arabinose supplementation in the gut lumen, we further explored the role of L-arabinose in disease progression in *Salmonella* infection. Surprisingly, when we infected BALB/c mice pretreated with streptomycin by *S. Tm* and provided L-arabinose-containing water for the duration of the experiment, no significant differences in survival rate (Fig. 4E) or body weight loss were observed (Fig. 4F). L-arabinose treatment did not have effect on the severity of colitis pathogenesis in streptomycin-pretreated mice. Mice from both groups exhibited multiple features of colitis with loss of epithelial crypts, inflammation, and edema (Figure S4E). Moreover, immunofluorescence showed that L-arabinose was unable to inhibit *S. Tm* to colonize cecal tissue at 96 h, although more bacteria could be observed in cecal contents when providing streptomycin-pretreated mice with water (Figure S4F). Similarly, we also observed comparable levels of PMN infiltration of the submucosa and epithelial layer between the L-arabinose-treated and untreated group, while more
PMN infiltration could be observed in the cecal contents of the untreated group at 96 h (Figure S4G). These results demonstrated that L-arabinose was unable to aggravate colitis caused by Salmonella infection in the absence of microbiota.

**L-arabinose reconstitutes the composition of Enterobacteriaceae in Salmonella-infected mouse gut.**

Exploitation of overlapping sugars by pathogenic bacteria and commensals is crucial for establishing and maintaining host colonization. Given that the virulence of S. Tm was not altered in vivo in the absence of microbiota, we supposed that S. Tm utilized L-arabinose to establish host colonization in competition with commensals. Inconsistent with the above speculation, L-arabinose-treated mice shed significantly less S. Tm throughout infection (day 1, 2, and 3 post-infection) in conventional mice (Figure S5A). Resident microbiota, such as commensal E. coli also consumes L-arabinose as a carbon source (19). Cometabolism of L-arabinose by Salmonella and commensals is likely to alter their ecological niche and abundance in the intestinal tract, which requires further exploration by 16S ribosomal DNA (rDNA) sequencing. In addition, mice were colonized by S. Tm at similar levels in systemic tissues, such as the spleen and liver (Figure S5B). The above observation suggested that Salmonella expansion was not responsible for more severe infection in L-arabinose-treated mice. Considering this, we hypothesized that the composition of the intestinal microbiota under L-arabinose treatment lowered fecal shedding as well as exacerbating systemic infection. Therefore, we profiled the effect of L-arabinose on microbiome composition using 16S rDNA sequencing. The Venn diagram showed that 90 operational taxonomic units (OTUs) were detected in the guts of uninfected mice (Fig. 5A). Moreover, when mice were infected with S. Tm, only 54 OTUs were identified in L-arabinose-treated mice, compared to 78 OTUs in the water-treated group.

Specifically, L-arabinose-treated mice presented the lowest α-diversity index in microbiota composition (Fig. 5B). The β-diversity was significantly different following L-arabinose treatment, indicating a shift in the overall gut microbiota composition (Stress < 0.063) (Fig. 5C). At phylum level, we observed a significant increase in the relative abundance of Proteobacteria in the L-arabinose-treated group compared with that in the water-treated group (Figure S6A and Table S1). Of this phylum, the family of Enterobacteriaceae was enriched in the L-arabinose-treated group (Fig. 5D and Table S1). However, L-arabinose-treated mice had a reduction in Salmonella (Fig. 5E and Table S2), which was consistent with the fecal shedding results (Figure S5A).

We also performed a linear discriminant analysis effect size (LEfSe) to identify specific taxa with varied abundance that would potentially be used as biomarkers. In total, we found 43 differentially abundant taxa among the three groups, all of which had a log linear discriminant analysis (LDA) score > 3. In addition, the LEfSe results validated that Enterobacter levels were significantly higher in the L-arabinose-treated group (Fig. 5E and S6B). Together, these data suggested that the family of Enterobacteriaceae bloomed due to L-arabinose supplementation in Salmonella-infected mice, limiting the abundance of Salmonella and reducing the diversity of the gut microbiota. Such an expansion of specific bacterial taxa
and rapid decrease in microbial diversity deteriorated to a state of dysbiosis, further exacerbating systemic infection.

**L-arabinose has no short-term effect on hyperglycemia by Salmonella infection.**

We noticed that L-arabinose mediates improved insulin sensitively and glucose uptake in both animals and humans (51, 52), suggesting that L-arabinose is a potential candidate for combating sucrose-related human pathologies. L-arabinose alleviates metabolic syndrome by regulating genes related to insulin sensitivity and lipid metabolism both in the liver and white adipose tissue (53). However, we revealed that L-arabinose exacerbated colitis and hepatic fibrosis upon *Salmonella* infection. In addition, the relative abundance of *Enterobacteriaceae* is significantly associated with the severity of type 2 diabetes mellitus (54, 55). Therefore, we profiled the impact of L-arabinose on *Salmonella*-infected hyperglycemic mouse model induced by streptozotocin (STZ). Mice were checked for hyperglycemia on day 5 post-STZ injection and then randomized for *Salmonella* infection (Fig. 6A). In accordance, the mice started to die 10 days post-infection, and all died within 14 days when water was provided *ad libitum*. In contrast, the mice supplied with L-arabinose drinking water started to die on day 8 post-infection, and all died within 12 days (Fig. 6B). The results demonstrated that L-arabinose also aggravated *Salmonella* infection in the STZ-induced hyperglycemic mouse model.

Given that chronic exposure to L-arabinose has been evidenced to exert anti-diabetic properties *in vivo*, we explored whether a high dose of L-arabinose supplementation ameliorates hyperglycemia and insulin resistance in *Salmonella*-infected mice. Conversely, no significant differences were observed in total serum cholesterol, triglyceride, low-density lipoprotein cholesterol levels, and blood glucose after 1 week of L-arabinose treatment (Fig. 6C).

**Discussion**

Pathogens utilize a variety of strategies to colonize in the gut and cause infection, such as scavenging nutrients, sensing chemical signals, competing with commensals, and regulating the expression of virulence genes (56). Several studies have elucidated the role of a certain carbon source in enteric infections (57, 58). In this study, we used *in vitro* and *in vivo* experiments to decipher the role of L-arabinose supplementation in *Salmonella* infection. Based on our data, we propose the following mechanistic model (Fig. 6D). In conventional mice, gut microbiota plays a central role in the prevention of *S. Tm* colonization. When mice are provided L-arabinose drinking water, the competition for L-arabinose between the resident microbiota and *S. Tm* supports *Enterobacteriaceae* expansion in the intestinal tract, causing microbiota dysbiosis and triggering serious inflammatory responses. In contrast, in the absence of microbiota, although L-arabinose is also a crucial nutrient for *S. Tm* initial expansion in the intestinal lumen, it represses the expression of SPI-1 through the cAMP-CRP pathway. As a result, L-arabinose ultimately does not influence host clinical symptoms.

**cAMP-CRP senses non-PTS sugars to mediate global regulation.**
It is widely accepted that cAMP-CRP induces catabolic pathways for growth on alternative substrates. Bacteria employ catabolite repression (CCR) systems for priority utilization of favorable carbon sources by inhibiting proteins involved in the use of less efficiently metabolized carbon sources. Although CCR is generally mediated by PTS, some non-PTS carbohydrates also enable to cause catabolite repression in *E. coli*, such as gluconate, lactose, and glucose-6-phosphate (59). In this study, we demonstrated that the non-PTS sugar, L-arabinose, decreased cAMP-CRP activity, which needs further exploration. cAMP-CRP is an archetypical global regulator that regulates the transcription of 7% of genes in *E. coli* (41). In addition, *crp* mutant causes a significant decrease in virulence compared with the WT, so it is listed as a live attenuated vaccine candidate (60). As expected, the Δ*cyaA* strain significantly repressed the expression of SPI-1 genes, as revealed by RNA-Seq. However, a previous study revealed that cAMP-CRP mediates silencing of *Salmonella* virulence by modulating the level of HilD at the post-transcriptional level (61). While this regulation occurs in the mid-logarithmic growth phase (non-permissive conditions), we found that cAMP-CRP mediates the activation of HilD in the early stationary phase (permissive conditions).

**YfiA is crucial for protein folding and degradation.**

The *yfiA* is highly expressed in the stationary phase, and its product binds to 30S subunits at the subunit interface to prevent dissociation of 70S ribosomes (43). It has been reported that factor-bound ribosome retains chaperoning activity and prevents aggregation of partially folded proteins (62). The protein aggregation has been shown to play a direct role in regulating gene expression in response to proteotoxic stress. These gene products, collectively termed heat shock proteins, include molecular chaperones (*e.g.*, DnaK) and ATP-dependent proteases (*e.g.*, Lon), which constitute the cellular network for proteins *de novo* folding and quality control under normal and stress conditions (63). A previous report demonstrated that Lon negatively regulates the expression of SPI-1 genes through degradation of HilD, as HilD dramatically accumulated in Lon-depleted cells (40, 64). As a result, we showed that faster degradation of HilD was responsible for L-arabinose-mediated SPI-1 repression.

**L-arabinose acts on cooperation between T3SS-1 expression and Salmonella expansion in the gut**

Utilizing a streptomycin-pretreated mouse model, we showed that L-arabinose was unable to alleviate the symptoms of *Salmonella* infection. Cells of the T3SS-1*+* phenotype grow slower than cells of the T3SS-1*−* phenotype, so virulence factor expression constitutes a considerable metabolic burden for pathogens (48). Moreover, short chain fatty acids (SCFAs) selectively slow the growth of T3SS-1*+* cells, ultimately decreasing their frequency and leading to population-level T3SS-1 repression (65). Since the pathogen has to “pay” a significant “price” for expressing T3SS-1, we speculate that T3SS-1*+* cells are repressed by L-arabinose, which constitutes a compensatory mechanism to benefit *S. Tm* initial expansion during host infection.

**Enterobacteriaceae expansion is a common feature of dysbiosis.**

Inhibition of pathogens colonization by the resident microbiota, a process that is also is called “colonization resistance”, plays an essential role in maintaining animal and human health (66). The
composition of the microbiota is profoundly affected by diet, changing the host’s susceptibility to infection (67). Differential dietary sugar availability influences the relationship between microbiota and enteric pathogens. For instance, galacturonic-acid utilization aids the initial expansion of *Citrobacter rodentium* (68). Interestingly, we found that L-arabinose limited the bloom of *S. Tm* as early as 24 h. This effect was ascribed to the specific composition of the microbiota induced by L-arabinose treatment. Moreover, the microbial dissimilation of L-arabinose promotes the intermediate metabolites production, such as acetate, lactate, propionate, and succinate (16, 21). Competition for L-arabinose as well as SCFAs shapes the environment of the colon in many ways, ultimately impacting disease outcomes. Supplementation with L-arabinose increased the relative abundance of *Enterobacteriaceae*, this phenomenon was caused by the special microbiota community of healthy BALB/c mice, as well as nutritional competition after *S. Tm* infection. While Proteobacteria and Firmicutes constitute the microbiota of healthy mice, Bacteroides were not detected in healthy mice by 16S rDNA sequencing. The unique bacterial community structure in BALB/c mice provides an explanation for this phenomenon, because the main phylum is determined to be Firmicutes, as reported by others (69). It has been shown that the gut microbiota is more unstable in BALB/c compared to C57BL/6J mice (69).

Proteobacteria bloom is a characteristic trait of an abnormal microbiota in the course of antibiotic therapy and dietary changes (70). In addition, microbiota dysbiosis is commonly observed in patients with inflammatory bowel disease, Crohn’s disease, and Ulcerative colitis, which are characterized by an increased relative abundance of facultative anaerobic bacteria (*e.g.*, *Enterobacteriaceae* and *Bacilli*) (71, 72). More importantly, dysbiosis disrupts the mucosal barrier, resulting in perpetuation of inflammation and carcinogenesis. Conversely, inflammatory disease conditions favor the overgrowth of pathogens and specific commensal species, in particular the family members of the *Enterobacteriaceae* (70, 73), which leads to a vicious cycle of enteric infections.

**L-arabinose poses a potential risk for diabetics with enteric infections.**

Sugar overconsumption is linked to a rise in the incidence of diseases such as diabetes, cardiovascular diseases, and gastrointestinal diseases. For example, high glucose intake exacerbates the pathogenesis of autoimmunity and inflammation (74). It has been reported that elevated consumption of the dietary component fructose worsen the DSS-induced or infectious colitis (75). In addition to these caloric simple sugars, a randomized-controlled clinical trial showed that non-nutritive sweeteners, saccharin and sucralose distinctly alter the stool microbiome and impair glycemic responses (76).

L-arabinose was approved as a food ingredient by the FDA GRAS (generally recognized as safe) Notification Program (GRN 782) in 2018. Supplementation with L-arabinose has been shown to significantly improve glucose intolerance and gut microbiota incoordination in diabetes (52, 77). However, we found that non-caloric L-arabinose exacerbated infectious colitis by altering microbiota composition. Some side effects, such as stomachache and diarrhea are associated with the addition of L-arabinose (9), which poses a potential risk for diabetics with bacterial infection. Since hyperglycemia affects both
microbiome structure and metabolism (78), we assume that the synergistic effect exerted by both L-arabinose and hyperglycemia further exacerbates colitis.

Materials and Methods

Bacterial strains, plasmids, media and culture conditions

Bacterial strains used in this study were listed in Table S3. Streptomycin resistance S. Tm 14028S-str was used for mouse infection. All derivatives were constructed using the λ Red recombinase system (79) and verified by PCR and sequencing. The plasmids were constructed using the ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China), and primers in this study were listed in Table S4. Strains were grown in LB medium supplemented with antibiotics as required. Solid media were prepared by the addition of 1.5% agar. Glucose, L-arabinose or D-galactose was added from 15% stocks prepared in distilled water. The final concentration of antibiotics in media was 100 µg/mL of ampicillin, 50 µg/mL of kanamycin, 17 µg/mL of chloramphenicol, 10 µg/mL of tetracycline or 50 µg/mL of streptomycin.

RNA isolation, sequencing and quantitative real-time PCR assay

The overnight culture was used to subculture in 50 mL fresh LB medium and incubated to early stationary phase (OD$_{600}$ = 2.0). The bacteria were harvested by centrifugation, and total RNA was isolated using the RNAprep Pure Cell/Bacteria Kit (Tiangen, Beijing, China). Mouse tissue RNA was extracted using TRIzol according to the manufacturer's instructions. RNA samples were quantified and reverse-transcribed with the random hexamers. Quantitative real-time PCR was carried out in a 20 µL of reaction mixture using ChamQ SYBR Color qPCR Master Mix (Vazyme). Fold changes in gene expression were calculated based on the $2^{-\Delta\Delta C_T}$ or $2^{-\Delta C_T}$ method.

One microgram of total RNA was used for library preparation and was loaded on an Illumina HiSeq 2000 instrument for sequencing. Cutadapt was used to process the technical sequences of fastq format. Clean data were aligned to the reference genome via software Hisat2 (v2.0.1). Differential expression analysis was performed using the DESeq2 Bioconductor package, and Padj of genes was set < 0.05 to detect differentially expressed genes.

Electrophoretic mobility shift assay

EMSA was performed as previously described (79). Briefly, the FAM-labeled promoter DNA was generated from S. Tm strain 14028S, and 10 nM promoter DNA was mixed with various amounts of protein in EMSA binding buffer (25 mM Tris-HCl, 50 mM KCl, 5 mM MgCl$_2$, 0.5 mM EDTA, and 10% glycerol, pH 8.0). Samples were incubated at 37°C for 30 min and separated on 7% acrylamide gels. DNA was visualized using a GE Amersham Imager 600.
Salmonella invasion assay

The ability of Salmonella to invade HeLa cells was determined using a gentamicin-protective assay as described previously with some modifications (79). Bacteria were washed twice and resuspended at a concentration of $1 \times 10^7$ CFU/mL in DMEM (without glucose) medium. Bacterial suspensions (200 µL per well) were then added directly on HeLa cells and incubated at 37°C for 1 h. Extracellular bacteria were killed by adding 1 mL DMEM containing 100 µg/mL gentamicin per well followed by 2 h incubation. HeLa cells were then disrupted by adding 1 mL of 1% (v/v) Triton X-100 per well. The percent invasion was calculated as the percentage of invasion rate in the experimental group compared to the control group (set to 100%).

Measurement of cellular ATP levels

Cultures of the WT or mutant strains were mixed with BacTiter-Glo™ reagent (Promega, Wisconsin, USA). Mix cultures briefly for 1 min and record the luminescence signal. The amount of ATP was calculated and normalized to the cell number.

Mouse experiments

Female 6-8-week-old BALB/c mice were used in this study. Mice were housed under specific pathogen-free conditions and maintained on a 12 h light/dark cycle. Food and water were provided ad libitum. Twenty milligrams of streptomycin were administered by oral gavage per day before infection when necessary. The infected mice were monitored daily for signs of clinical illness.

Mice were infected by oral gavage with 100 µL of PBS containing approximately $1.5 \times 10^7$ CFU of S. Tm. Sterile drinking water containing 300 mM L-arabinose was provided ad libitum during the whole infectious process. The survival of the mice was observed daily after infection.

In competitive infections, two competitive S. Tm strains at a ratio of 1:1 were given via oral gavage at a combined final concentration of $1.5 \times 10^7$ CFU/mouse. For CFU determinations, the spleen and liver were collected and plated in ten-fold serial dilutions at day 4 post-infection. To determine the bacterial numbers in the feces, fecal pellets were collected individually from mice, weighed, homogenized in cold PBS, and plated at serial dilutions onto LB agar. Ceca and colons were harvested, fixed, and processed for H&E staining and immunohistochemistry. Mice were sacrificed on day 4 post-infection, and colon content and tissue were collected for 16S rDNA sequencing and RT-qPCR analysis.

Streptozotocin-induced diabetic mouse model

Single, high dose of STZ was utilized to induce type 1 diabetic model in C57BL/6 male mice (80). To induce hyperglycemia, STZ was diluted freshly in 50 mM sodium citrate buffer (pH 4.5) and injected intraperitoneally at 200 mg/kg. Provide 10% sucrose drinking water until experimental day 3 to avoid post-procedural hypoglycemia. Five days post-injection, fasting blood glucose was tested from the tail vein by using One Touch Basic Blood Glucose Monitoring System (Roche). Mice (fasting blood glucose ≥
150 mg/dL) were selected for subsequent bacterial infection. For *Salmonella* infection, mice were infected by oral gavage with 100 CFU of strain 14028S-str, and serum was collected on day 12.

**Lipocalin-2 quantification**

Fresh stool samples from the mice were collected in tubes and frozen in lipid nitrogen immediately. Feces were homogenized in 1 mL of sterile PBS and supernatants were collected to detect lipocalin-2 by Mouse NGAL ELISA Kit (Proteintech, Chicago, IL, USA).

**Colonic epithelial barrier permeability measurement by FITC-dextran**

Mice were received 150 µL of FITC-Dextran (4 kDa) suspension (80 mg/mL) by gavage on day 4 of infection. After 3 h, blood was collected and centrifuged at 4°C. The fluorescence signal of serum was quantified at 485/525 nm (excitation/emission).

**Bioluminescent imaging**

For measuring bioluminescence in LB medium, luciferase-expressing plasmids were transferred into *S. Tm* strain 14028S by electroporation. The luminescence of *Salmonella* was quantified in 96-well plates using thenergyHT equipment. For *in vivo* BLI, mice were infected with 1.5×10⁷ CFU of *S. Tm* strain 14028S-str, carrying luciferase-expressing plasmids. Mice were anesthetized and bioluminescence was quantified using the IVIS2000 instrument and Living Image software (Perkin Elmer) at different time points. Radiance was measured using the region of interest (ROI) tool.

**Extraction of genomic DNA and 16S rDNA sequencing**

Total genomic DNA of fecal samples were extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using the forward primer (CCTACGGGRBGCASCAGKVRVGAAT) and the reverse primer (GGACTACNVGGGTWTCTAATCC). Next-generation sequencing was conducted on an Illumina Novaseq platform. Raw sequence data were quality-filtered, followed by VSEARCH clustering (1.9.6) for OTUs clustering. The OUTs were classified taxonomically using RDP classifier (Ribosomal Database Program) and calculate the relative abundance of microbiota at different levels. Shannon index was obtained using a random sampling method. The NMDS was based on the distance between the matrix Brary-Curtis to display β-diversity. The LEfSe scores measure the consistency of differences in relative abundance between taxa in the groups, with a higher score indicating higher consistency.

**Statistical analysis**

All the data were collected from more than three independent experiments. Data were analyzed using the GraphPad Prism 8.0 software (GraphPad Software). Statistically significant differences were examined using two-tailed Student’s *t* test or Mann-Whitney test to derive the significance of differences between two groups. One-way analysis of variance (ANOVA) test was used for multiple comparisons. Log-rank test
was used for comparing survival distributions between groups. Shannon index was compared with the Kruskal-Wallis test. $P<0.05$ was considered to be significant.

**Declarations**

**Ethics approval and Consent to participate**

All animal procedures were approved by Shanghai Jiao Tong University School of Medicine, and this study was carried out in strict accordance with the National Research Council Guide for Care and Use of Laboratory Animals [SYXK (Shanghai 2018-0027)]. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

**Competing interests**

The authors declare no competing interests.

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**Authors' contributions**

J.Y., J.L. and Y.-F.Y. conceived the project and designed the experiments. J.Y., H.T., N.Z., Z.W., W.H., J.N., and D.W. carried out the experiments and analyzed data. J.Y. wrote the initial draft of the manuscript. J.Y., J.L., and Y.-F.Y. reviewed and edited the manuscript to its final form. All authors read, commented on, and approved this manuscript.

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Not applicable.
References


Figures
Figure 1

L-arabinose exacerbates *Salmonella* infection in conventional mice.

(A) Survival curve, (B) Body weight loss of conventional mice infected with S. Tm by providing 300 mM L-arabinose (L-ara) drinking water. Log-rank test was used for comparing survival distributions between groups. (C) H&E-stained colons of mice. The colons were fixed and embedded in paraffin and stained...
with H&E. Images were taken at ×200 magnification (scale bars, 200 μm). (D) Colon lengths of mice. Mice were sacrificed on day 4, and colon lengths were measured. (E) Gut inflammation as measured by Lipocalin-2 ELISA. Feces were harvested on day 4 and homogenized in PBS for detecting Lipocalin-2. (F) Intestinal permeability determined by measuring FITC-Dextran in serum. *, $P < 0.05$, **, $P < 0.001$, Student’s $t$ test. (G) AB-PAS stained images of colonic sections showing the mucus layers (arrows). Images were taken at ×200 magnification (scale bars, 200 μm).
Figure 2

cAMP-CRP is required for repression of SPI-1 genes by addition of L-arabinose.

The transcriptional levels of (A) hilD and (B) hilA in LB medium supplied with 0.2% glucose (Glu), L-arabinose (L-ara) or D-galactose (D-gal). Bacteria were grown to OD$_{600}$ ~ 2.0 and harvested for total RNA isolation. The transcriptional levels of the candidate genes were determined by qPCR with the methods of 2$^{-\Delta \Delta Ct}$. (C) Western blot of SipB under different sugar supplementation conditions. The chromosome Flag-fused sipB strain was cultured in LB medium by adding 0.2% sugars, and SipB was determined by the anti-Flag antibody (α-Flag). DnaK was determined by the anti-DnaK antibody (α-DnaK) as an internal control. (D) Relative bacterial invasion rates in the presence of different sugars. HeLa cells were cultured with DMEM (without glucose). Bacteria were cultured to OD$_{600}$ ~ 2.0 and infected HeLa cells at an MOI of 100. (E) Levels of phosphorylated and unphosphorylated EIIA$^{Glc}$ protein. The flag-tagged crr strain was grown in LB medium with 0.2% sugars and harvested for preparation of lysate. Proteins were separated on a Phos-tag gel and detected with anti-Flag antibody (α-Flag). Amounts of the phosphorylated EIIA$^{Glc}$ were quantitated using an image analyzer, and ratios of the amount of phosphorylated compared with the unphosphorylated protein are indicated below the protein bands. (F) Western blot of CRP under different sugars supplementation conditions. The chromosome Flag-fused crp strain was cultured in LB, cells were collected at OD$_{600}$ ~ 2.0 and the CRP was determined by the anti-Flag antibody (α-Flag). (G) The transcriptional levels of hilD and hilA in ΔaraΔcrp. ΔaraΔcrp cells were cultured in LB medium by adding 0.2% L-arabinose. Bacteria were harvested for RNA isolation and qPCR. **, $P < 0.01$, ***, $P < 0.001$, ANOVA analysis.
cAMP-CRP directly activates yfiA to regulate HilD degradation.

(A) Volcano plot showing differentially regulated genes in a ΔcyAA versus the WT strain. The x-axis is the log₂ scale of the fold change of gene expression, and y-axis is the minus log₁₀ scale of q values. The red dots represent significantly up-regulated genes with at least two-fold change, while the blue dots
represent significantly down-regulated genes with at least two-fold change. (B) Heatmap of differentially expressed genes in ΔcyA versus the WT strain. A cluster analysis was performed using the log₂ (fkm+1) value and standardized by Z-score. (C) EMSA of cAMP-CRP binding to yfiA promoter. A FAM-labeled yfiA promoter (positions -186 to +51) was used in binding reactions with or without cAMP (0.2 mM) and different CRP concentrations, as shown. (D) Schematic diagram of gfp translational fusion constructs driven by WT (P_{yfiA(WT)}) or mutant (P_{yfiA(Mu1)}) yfiA promoter. (E) GFP fluorescent signals from the plasmids carrying yfiA-gfp fusion were monitored in WT (WT/P_{yfiA(WT)}, WT/P_{yfiA(Mu1)}) and Δcrp (Δcrp/P_{yfiA(WT)}, Δcrp/P_{yfiA(Mu1)}). (F) Stability of HilD in ΔyfiA and the WT strain. The WT or the mutant strain was transformed with pQE80-hilD plasmid. Degradation of HilD was determined by western blot using an anti-His antibody (α-His). (G) Invasion assays of WT, ΔyfiA and ΔyfiA::yfiA strain in HeLa cells. ***, P < 0.001, ANOVA analysis.
L-arabinose does not influence disease progression in a streptomycin-pretreated mouse model.

(A) Abdominal luminescence driven by sicA promoter from 2 representative mice from 8 to 48 h post-infection. Streptomycin-pretreated mice were infected with S. Tm harboring pACYC184-sicA-lux, the promoter signals were monitored by BLI system. (B) Quantification of luminescence signals driven by...
siCPromoter using the living Image software. Bioluminescence values were expressed as the total radiance (photons/second). (C) Abdominal luminescence driven by \textit{cat} promoter from 2 representative mice from 8 to 48 h post-infection. (D) Quantification of luminescence signals driven by \textit{cat} using the living Image software. Data are compared at the different time as the means ± SD. *, \( P < 0.05 \), **, \( P < 0.01 \), by two-tailed Mann-Whitney test. (E) Survival curve, (F) Body weight loss of streptomycin-pretreated mice infected with \textit{S. Tm} by providing 300 mM L-arabinose (L-ara) drinking water. Log-rank test was used for comparing survival distributions between groups.
The relative abundance of *Enterobacteriaceae* is enriched in L-arabinose-treated mice.

(A) The Venn diagrams showing OTUs detected in uninfected, L-arabinose-treated (Infected/L-ara), and water-treated (Infected/water) mice. Mice were fed with 300 mM L-arabinose drinking water for 4 days followed by *S. Tm* infection. Water-treated groups were not fed with any sugar in the drinking water. (B) The α-diversity (Shannon index) of 16S rDNA sequencing among three groups. Statistical significance was calculated using Kruskal-Wallis. (C) The β-diversity (non-metric multidimensional scaling, NMDS) of 16S rDNA sequencing among three groups. (D) Relative OTUs abundance of taxonomic distributions at family level. (E) Relative abundance of *Enterobacter* and *Salmonella* in the gut microbiota. Metastats were used to determine differentially abundant bacterial taxa between groups.
Figure 6

L-arabinose is unable to alleviate symptoms of hyperglycemia in *Salmonella*-infected mice.

(A) Experimental design of *Salmonella* challenge study in STZ-induced hyperglycemia model. (B) Survival curve of STZ-induced mice infected with *S. Tm* by 300 mM L-arabinose (L-ara) addition. Log-rank test was used for comparing survival distributions between groups. (C) LDL-c, serum glucose, TG level, and
cholesterol of serum at day 12 post-infection. Data are presented as the means ± SD. Ns, $P > 0.05$, Student’s $t$ test. (D) Working model displays the role of L-arabinose supplementation in *Salmonella* infection. The colonization resistance mediated by competition between commensal microbiota and *S*. Tm protects the host from infections. However, L-arabinose supplementation causes an expansion of *Enterobacteriaceae*, disturbing colonization resistance and leading to microbiota dysbiosis. Finally, such intervention caused by L-arabinose worsen the severity of systemic infection in typhoid mouse model. For bacteria, *S*. Tm achieves a balance between virulence and metabolic gene expression in the gut when microbiota is absent.

**Supplementary Files**

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- TableS1.xlsx
- TableS2.xlsx
- TableS3.xlsx
- TableS4.xlsx
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