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Research Article

RESEARCH ARTICLE

Lactic acid bacteria decrease *Salmonella enterica* Javiana virulence and modulate host inflammation during infection of an intestinal epithelial cell line

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One sentence summary: Lactic acid bacteria (LAB) decrease S. Javiana virulence and modulate host inflammatory response to infection.

ABSTRACT

Salmonella enterica Javiana is a leading cause of severe foodborne Salmonellosis. Despite its emergence as a major foodborne pathogen, little is known of how S. Javiana interacts with intestinal epithelial cells, or of potential methods for ameliorating the bacterial-host interaction. Using cell-based adhesion, invasion and lactate dehydrogenase release assays, we observed an invasive and cytotoxic effect of S. Javiana on intestinal epithelial cells. We assessed the effect of probiotic species of lactic acid bacteria (LAB) on the S. Javiana-host cell interaction, and hypothesized that LAB would reduce S. Javiana infectivity. *Salmonella enterica* Javiana invasion was significantly impaired in host cells pre-treated with live *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. In addition, pre-exposure of host cells to live *L. acidophilus*, *L. rhamnosus* and *L. casei* reduced S. Javiana-induced cytotoxicity, while heat-killed LAB cultures had no effect on S. Javiana invasion or cytotoxicity. qRT-PCR analysis revealed that S. Javiana exposed to *L. acidophilus* and *L. rhamnosus* exhibited reduced virulence gene expression. Moreover, pre-treating host cells with LAB prior to S. Javiana infection reduced host cell production of inflammatory cytokines. Data suggest a potential protective effect of *L. acidophilus*, *L. rhamnosus* and *L. casei* against intestinal epithelial infection and pathogen-induced damage caused by S. Javiana.

Keywords: *Salmonella enterica* Javiana; lactic acid bacteria; probiotics; intestinal epithelial cells; invasion; cytotoxicity

INTRODUCTION

Salmonella is a Gram-negative, facultatively anaerobic bacillus and member of the *Enterobacteriaceae* family. The *Salmonella* genus is comprised of two species, *Salmonella bongori* and *Salmonella enterica*, the latter of which contains most medically-relevant strains. *Salmonella enterica* is a highly ubiquitous species containing over 2600 serovars that can be divided into typhoidal and non-typhoidal *Salmonella* (NTS) (Gal-Mor, Boyle and Grassl 2014). Typhoidal serovars (*S. enterica* Typhi and *S. enterica* Paratyphi) cause life-threatening systemic disease, while most NTS serovars cause serious gastroenteritis and other acute infections in humans and animals. NTS strains are the third leading cause of bacterial foodborne illness globally (Majowicz *et al.* 2010), and cause nearly 94 million illnesses and 155 000 deaths annually worldwide (Kirk *et al.* 2015). Each year in the U.S., *Salmonella* causes an estimated 1.2 million illnesses, 23 000 hospitalizations and 450 deaths (Centers for Disease Control and Prevention 2018), and the majority of these infections are attributed to consumption of NTS strains in contaminated food products, including meat, poultry, eggs, cheese, seafood and produce (Jackson *et al.* 2013). Although more than 2400 serovars of NTS have been identified, most cases of *Salmonella*-induced gastroenteritis are attributed to five serovars: S. Typhimurium, S. Enteriditis, S. Newport, S. Javiana and S. Heidelberg (Boore *et al.* 2015).

Of the leading strains of NTS, S. Javiana is the fourth most common (Boore *et al.* 2015), but is one of the least characterized in terms of its interaction with the host intestinal epithelium.

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The majority of S. Javiana strains contain *Salmonella* Pathogenicity Island-1 (SPI-1) and SPI-2 encoded virulence genes (Allard *et al.* 2013; Mezal, Stefanova and Khan 2013) commonly found in other gastroenteritis-associated NTS serovars, and which enable colonization and invasion of the intestinal epithelium, and intracellular survival, respectively (Zhou 2001; Abrahams and Hensel 2006). In addition, genomic analyses have revealed that S. Javiana strains possess the genes *pltA*, *pltB* and *cdtB*, which together encode the cytolethal distending toxin (CDT), a virulence factor more commonly associated with infection by the typhoidal strain S. Typhi than NTS strains (Mezal, Stefanova and Khan 2013), and which promotes host cell invasion, cell cycle arrest, DNA damage and systemic spread (Williams *et al.* 2015; Miller *et al.* 2018). However, few studies have examined the interaction of S. Javiana with intestinal epithelial cells during infection or potential ways to ameliorate the host-pathogen interaction.

NTS are a public health concern not only due to the frequency of infection, but also because of the emergence of antimicrobial resistant strains. In the U.S., there are an estimated 100 000 cases of drug-resistant Salmonellosis each year, over 66 000 of which are caused by multidrug-resistant NTS (Centers for Disease Control and Prevention 2013). Not surprisingly, resistance is rising fastest among the leading NTS strains, including S. Javiana. In 2002 the CDC reported no resistance to commonly used antibiotics among S. Javiana isolates from an outbreak (Centers for Disease Control and Prevention 2002), but more recent studies indicate emergence of ampicillin-, tetracycline- (Mezal, Stefanova and Khan 2013), sulfisoxazole- (Micallef *et al.* 2012), gentamicin-, streptomycin- and kanamycin-resistance (Santos *et al.* 2007; Mezal, Stefanova and Khan 2013; Angelo *et al.* 2016; Nair, Venkitanarayanan and Johny 2018) among S. Javiana isolated from clinical, food and environmental sources. Given the high incidence of foodborne Salmonellosis and rise in drug resistance among NTS serovars, there is urgent need for discovery and development of antibiotic alternatives for prevention or treatment of *Salmonella* infections, including those caused by S. Javiana.

Probiotics, which are viable microorganisms that upon ingestion have beneficial effects on the host, have gained widespread attention for their anti-infective properties and potential use as non-antibiotic prophylactic or therapeutic agents (Pereira *et al.* 2018). While a wide variety of bacteria and yeast are reported to have probiotic properties (Surendran Nair, Amalaradjou and Venkitanarayanan 2017), species of lactic acid bacteria (LAB) are well-documented for exhibiting antimicrobial and anti-virulence effects against gastrointestinal (GI) pathogens (Fuller 1992) and for exerting immunoregulatory and homeostatic effects on the host (Lievin-Le Moal and Servin 2014; George *et al.* 2018; Zhang *et al.* 2018). Indeed, *in vitro* and *in vivo* studies have shown that LAB can limit infectivity of GI pathogens, including NTS serovars (Chen *et al.* 2012; Lievin-Le Moal and Servin 2014; Dutra *et al.* 2016; Muyyarikkandy and Amalaradjou 2017). However, since the anti-infective properties of probiotics are often unique to specific strains of probiotics and pathogens (Chen *et al.* 2012; Campana, van Hemert and Baffone 2017), and since to our knowledge, no studies have examined the impact of probiotics on intestinal epithelial cell infection by S. Javiana, whether LAB might affect S. Javiana interaction with intestinal epithelial cells is not known. Therefore, we sought to use a cell-based infection model to examine the interaction of S. Javiana with intestinal epithelial cells and to investigate the potential for probiotic LAB strains to modulate S. Javiana infection. Here, we report that S. Javiana has an invasive phenotype and cytotoxic effect on the human HT29-MTX intestinal epithelial cell line, and that the LAB species *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Lactobacillus casei* can reduce S. Javiana invasion, limit pathogen-induced cell damage, alter S. Javiana virulence gene expression and modulate the host cell inflammatory response to infection.

MATERIALS AND METHODS

Bacterial strains and growth conditions

All bacterial strains used in this study are listed in Table 1. *Salmonella enterica* Javiana and *Escherichia coli* were grown in Luria Bertani (LB) broth, while LAB species *L. acidophilus*, *L. rhamnosus*, *L. casei*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were grown in deMann-Rogosa-Sharpe (MRS) broth. All bacterial cultures were stored at −80C with addition of 20% (vol/vol) glycerol. Prior to use in experiments, cultures were subcultured onto either LB agar or MRS agar and individual colonies were grown overnight either in LB broth in a shaking incubator at 37°C or in MRS broth in a static incubator at 37°C.

Cell culture

The mucus-secreting human colonic cell line HT29-MTX-E12 (HT29-MTX) was purchased from Sigma Aldrich. HT29-MTX cells were cultured in Roswell Park Memorial Institute 1640 (RPMI) medium containing 10% FBS (v/v), 1% GlutaMAX (Life Technologies), 1% HEPES buffer and 1% of a 100X penicillin-streptomycin (VWR). Cells were incubated at 37°C in a humidified 5% (v/v) CO_2 atmosphere and used between passages 10 and 20. Cells were seeded into 24-well plates (for adhesion, invasion, cytotoxicity and cytokine assays) at a density of 4.0×10^4 cells per well or into 6-well culture plates (for gene expression studies) at a density of 1.7×10^5 cells per well. Cell medium was changed every two days and medium without antibiotic was used for the last medium change prior to infection assays. Infections were performed on confluent monolayers at 14–21 days post-seeding, to ensure that HT29-MTX cells had reached maturity (Lesuffleur *et al.* 1993).

Bacterial adhesion, invasion and intracellular survival assays

All infection assays were performed in RPMI growth medium, at 37°C in 5% CO_2 atmosphere. To assess adhesion and invasion of S. Javiana or *E. coli* (non-pathogenic negative control) to HT29-MTX monolayers, overnight bacterial cultures were washed and resuspended in RPMI medium and then added to HT29-MTX monolayers grown on sterile coverslips in 24-well plates at multiplicity of infection (MOI) of 10. For adhesion assays, at 1 h p.i., monolayers were washed three times with Dulbecco's PBS (D-PBS, Lonza) to remove non-adherent bacteria, then incubated with 0.01% Triton X-100 (Sigma Aldrich) for 5 min to dislodge attached bacteria (Burkholder and Bhunia 2010), and adherent bacteria were enumerated by plating onto LB agar. For invasion assays, at 2 h p.i. infected monolayers were treated with gentamicin (100 μg/mL) for 30 min to kill extracellular bacteria, then washed three times with D-PBS and lysed with 0.1% Triton-X (Burkholder and Bhunia 2009; Burkholder and Bhunia 2010). Intracellular bacteria were enumerated by plating cell lysates onto LB agar.

To examine the impact of LAB pre-treatment on S. Javiana adhesion or invasion, overnight LAB cultures were washed and resuspended in RPMI medium, then added to HT29-MTX monolayers grown on sterile coverslips in 24-well plates at multiplicity

Table 1. Bacterial strains used in this study.

Bacterium	Strain description	Source or reference
Salmonella enterica Javiana	CFSAN[1] 0 01992, human stool isolate	Obtained from Dr. Marc Allard (U.S. FDA) (2)
Escherichia coli	DUP-101 strain, ATCC[2] 51 739	ATCC
Lactobacillus acidophilus	ARS NRRL[3] B1910	Our collection
Lactobacillus casei	KCTC[4] 3109	Our collection
Leuconostoc mesenteroides	ARS NRRL B-1118, isolated from olives	Our collection
Lactobacillus plantarum	ARS NRRL B-4496, ATCC 14 917, isolated from pickled cabbage	Our collection
Lactobacillus rhamnosus GG	ATCC 53 103, human stool isolate	Our collection (15)

[1]Center for Food Safety and Applied Nutrition
[2]American Type Culture Collection
[3]Agricultural Research Service (ARS) Northern Regional Research Laboratory (NRRL) Culture Collection
[4]Korean Collection Type Cultures

of exposure (MOE) of 10. At 1 h post-LAB exposure, non-adherent LAB were removed by washing HT29-MTX cells with D-PBS, S. Javiana was added to cell medium at MOI 10 and infected cells were incubated for 1 h. At 1 h post-infection, S. Javiana adhesion was assessed, and at 2 h post-infection S. Javiana invasion was determined as described above. Adherent and intracellular S. Javiana were enumerated on LB agar, which did not support the growth of any LAB tested (data not shown).

For invasion assays using heat-killed LAB (HK-LAB), overnight LAB cultures were washed, resuspended in sterile water and autoclaved at 121°C for 15 min to kill the bacteria (Wagner *et al.* 2000). Aliquots of autoclaved cultures were plated on MRS agar to assess loss of viability, and Gram stains were prepared to ensure presence of bacterial cells in the autoclaved suspensions. HK-LAB were pelleted, resuspended in RPMI medium and added to HT29-MTX monolayers at MOE 10. At 1 h post-exposure to HK-LAB, HT29-MTX cells were washed, infected with S. Javiana (MOI 10) and S. Javiana invasion was assessed as described above.

To assess the effect of LAB pre-treatment on the number of intracellular S. Javiana present at 6 h p.i., HT29-MTX cells were either infected with S. Javiana alone (MOI 10) or pre-treated with live *L. acidophilus*, *L. rhamnosus* or *L. casei* (MOE 10) for 1 h prior to S. Javiana infection. At 2 h p.i., gentamicin (100 μg/ml) was added to the cell media to kill extracellular bacteria. At 6 h p.i., epithelial cells were washed three times with D-PBS, lysed with 0.1% Triton-X, and intracellular S. Javiana were enumerated by plating on LB agar.

Cytotoxicity assays

A lactate dehydrogenase (LDH) release assay (Thermo Fisher Scientific) was used to quantify host cell damage induced by S. Javiana (Burkholder and Bhunia 2009). HT29-MTX cells were grown in 24-well plates and infected with S. Javiana or *E. coli* K12 (non-pathogenic control) as described above for adhesion and invasion assays. Uninfected cells were used as a negative control and cells treated with 0.1% Triton-X (TX) served as a positive control. At 2 h p.i., HT29-MTX supernatants were collected and centrifuged (800 x g for 5 min) to remove bacterial and eukaryotic cells. A 100 ul aliquot of each sample was dispensed into triplicate wells of a 96-well plate and LDH activity was determined spectrophotometrically per manufacturer's protocol, using the formula: % Cytotoxicity of sample = $((Abs_{TX}–Abs_{sample})/(Abs_{TX}–Abs_{Uninfected}))*100$.

To assess the effect of individual LAB strains on S. Javiana-induced cytotoxicity, overnight LAB or *E. coli* cultures were added to HT29-MTX monolayers at MOE of 10 as described above. At 1 h post- exposure, non-adherent bacteria were removed by washing HT29-MTX cells with D-PBS, S. Javiana was added to cell medium at MOI 10 and infected cells were incubated for 2 h. At 2 h p.i., HT29-MTX cell supernatants were collected, LDH was quantitated and % cytotoxicity calculated as detailed above. For cytotoxicity assays using HK-LAB, overnight LAB cultures were autoclaved at 121°C for 15 min as described above, and then resuspended in RPMI medium and added to HT29-MTX monolayers at MOE 10 for 1 h prior to infection with S. Javiana. In separate experiments, we also performed an LDH assays with individual LAB strains, in absence of S. Javiana, to ensure that LAB alone did not have cytotoxic effects on HT29-MTX cells (Fig. S1, Supporting Information).

Salmonella viability assay

To assess the impact of LAB on S. Javiana viability, overnight cultures of S. Javiana alone or S. Javiana plus individual LAB strains were diluted 1:50 in RPMI and incubated at 37°C in 5% CO_2, to mimic the growth conditions and bacterial concentrations used in infection assays. At 0, 2, 4 and 6 h post-inoculation, aliquots were obtained and plated on LB agar (which allowed enumeration of S. Javiana but not LAB) to quantify S. Javiana viability, and on MRS agar (which allowed enumeration of LAB but not S. Javiana) to confirm viability of LAB strains (MRS data not shown).

Analysis of the effect of LAB on *Salmonella* virulence gene expression

The effect of *L. acidophilus*, *L. rhamnosus* and *L. casei* on expression of S. Javiana virulence genes *invA*, *prgH*, *pltA* and *cdtB* was determined by qRT-PCR. These strains of LAB were chosen for these experiments because they were the strains that impaired S. Javiana invasion and S. Javiana-induced cytotoxicity. To mimic conditions used in infection assays, gene expression studies were performed in RPMI medium in the presence of HT29-MTX cells. Briefly, HT29-MTX cells grown in 6-well plates were pre-treated with or without individual strains of *L. acidophilus*, *L. rhamnosus* or *L. casei* (MOE 10). At 1 h post-LAB exposure, S. Javiana was added to the cell medium at MOI 10. At 2 h p.i., HT29-MTX supernatant and monolayers were harvested (to collect extracellular and intracellular S. Javiana), samples were pelleted and pellets were resuspended in Qiagen RNeasy mini kit buffer RLT containing β-mercaptoethanol and added to tubes containing 0.2 mm Rnase-free stainless steel beads (Next Advance). Samples were homogenized using a Bullet Blender cell disruptor

Table 2. Primers used in this study.

Primer[a]	Sequence (5′–3′)	Source
cdtB-F primer	ATGTCTTGCGTCCGACAACT	This study[b]
cdtB-R primer	CGTGCGCTGTCAGAAAAACA	
pltA-F primer	TTTACCAGACCTGTTGCGCT	This study
pltA-R primer	AGCTTGCTCCCATCCATCAC	
invA-F primer	CGCGCTTGATGAGCTTTACC	This study
invA-R primer	TCGCTTAACAAACGCTGCAC	
prgH-F primer	GGGCGCTCGATGATGTAGAA	This study
prgH-R primer	TGGCCTGGGCTCATTTTGAT	
16S-F primer	GGCGCATACAAAGAGAAGCG	This study
16S-R primer	CTCCAATCCGGACTACGACG	

[a]F, forward; R, reverse

[b]All primer sequences based on the *Salmonella enterica* subsp. *enterica* serovar Javiana strain CFSAN001992 chromosomal genome sequence (GenBank accession no. NC_02 0307.1)

(Next Advance) and mRNA was prepared from the homogenate using the Qiagen RNeasy kit according to the manufacturer protocol. Samples were treated with RNase-free DNase I (Qiagen) to remove remaining host cell and bacterial DNA and were analyzed for purity using a NanoDrop spectrophotometer. The resulting RNA was stored at −80°C. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) and samples were used for RT-PCR with PowerUp Sybr Green Master Mix (Life Technologies) and the primer sequences listed in Table 2. Real time detection and relative quantitation of transcripts were achieved with the StepOne Plus Real-Time PCR System (Life Technologies). Prior to performing qRT-PCR, all primers were validated against DNA from *S. Javiana*, *L. acidophilus*, *L. rhamnosus*, *L. casei* and HT29-MTX cells to ensure that they amplified only *S.* Javiana DNA, and that there was no non-specific amplification of LAB or host cell DNA.

The effect of LAB on *S.* Javiana gene expression was determined using the comparative quantification ($\Delta\Delta C_T$) method (Livak and Schmittgen 2001; Tranchemontagne *et al.* 2016). Briefly, the $\Delta\Delta C_T$ method compares the threshold cycle (C_T) from an experimental sample (from LAB-exposed *S.* Javiana) with both a calibrator sample (from *S.* Javiana not exposed to LAB) and a normalizer (*S.* Javiana-specific 16S rRNA housekeeping gene measured in experimental and calibrator samples). The ΔC_T value, representing the difference in threshold cycle between the target and normalizer genes, was determined by subtracting the C_T value of the 16S rRNA gene from the C_T values for each target gene (*invA, prgH, pltA*, or *cdtB*). The $\Delta\Delta C_T$ value was derived from the subtraction of the ΔC_T of the calibrator sample from the ΔC_T of the experimental sample. $2^{-\Delta\Delta CT}$ was expressed as the n-fold difference in gene expression in the experimental sample compared to the calibrator sample at each time-point tested. Genes exhibiting greater than 3-fold change in expression were considered to be altered by LAB exposure.

Cytokine assays

To determine whether LAB exposure would alter host cell cytokine secretion in response to *S.* Javiana infection, HT29-MTX monolayers were grown in 24-well plates and were either uninfected, exposed to *S.* Javiana alone (MOI 10), or pre-treated with individual LAB (MOE 10) for 1 h followed by *S.* Javiana infection (MOI 10). At 2 h p.i., gentamicin (100 μg/mL) was added to the infection medium to kill extracellular bacteria, and monolayers were incubated for an additional 4 h to allow for secretion of cytokines. Infected cell supernatants were collected and analyzed for cytokines using the Human Inflammation Quanitbody array (RayBiotech) according to manufacturer protocol. Completed arrays were shipped to the manufacturer for scanning and cytokine quantitation. The limit of detection for cytokines in this assay is 3 pg/ml (RayBiotech).

Statistical analysis

Differences between treatments were assessed by analysis of variance (ANOVA) using R, version 2.15 (R Development Core Team 2012). Any significant ANOVA tests were further analyzed using Tukey's post hoc pairwise comparisons. We used an alpha value of 0.05, so P values of <0.05 were considered significant. All error bars represent standard deviations.

RESULTS

Adhesion, invasion and cytotoxic effect of *S.* Javiana during infection of HT29-MTX epithelial cells

Few studies have examined the interaction of *S.* Javiana with intestinal epithelial cells. Therefore, we used the human colonic HT29-MTX intestinal epithelial cell line (Gagnon *et al.* 2013) to examine *S.* Javiana adhesion, invasion and cytotoxic effect during host cell infection. Nonpathogenic *E. coli* was used as a control that, although moderately adhesive, exhibits little invasion or cytotoxicity. While *S.* Javiana adhered to HT29-MTX cells at levels similar to *E. coli* (Fig. 1A), as expected, *S.* Javiana exhibited significantly greater invasion than *E. coli* into host cells (Fig. 1B). LDH release assays revealed significantly greater cytotoxic effect of *S.* Javiana compared to the nonpathogen (Fig. 1C). Together, these findings confirm that, similar to other gastroenteritis-producing strains of NTS, *S.* Javiana interacts with, invades and damages epithelial cells during infection (Wallis and Galyov 2000; Bergeron *et al.* 2009; Burkholder and Bhunia 2009).

LAB reduce *S.* Javiana invasion and *S.* Javiana-induced cytotoxicity during epithelial cell infection

Previous reports from our work and others have shown that LAB can hinder the interaction of *Salmonella* with host cells and ameliorate *Salmonella*-induced cell or tissue damage (Burkholder and Bhunia 2009; Chen *et al.* 2012; Campana, van Hemert and Baffone 2017; Muyyarikkandy and Amalaradjou 2017; Liu *et al.* 2018). Therefore, we sought to examine, in our HT29-MTX infection model, the effect of LAB species commonly used as probiotics (Burkholder and Bhunia 2009; Campana, van Hemert and Baffone 2017; Choi *et al.* 2018) on *S.* Javiana adhesion, invasion and *S.* Javiana-induced cytotoxicity. We performed adhesion, invasion and LDH release assays in which host cells were either untreated or pre-exposed to *L. acidophilus*, *L. mesenteroides*, *L. rhamnosus*, *Lactobacillus plantarum* or *L. casei* (MOE 10) for 1 h prior to infection with *S.* Javiana (MOI 10). All LAB strains bound to the HT29-MTX cells and were adherent at the time of *S.* Javiana infection, with *L. acidophilus*, *L. rhamnosus* and *L. casei* exhibiting greatest host cell adhesion (Fig. S2, Supporting Information). While there was no effect of any LAB strain on *S.* Javiana adhesion to HT29-MTX cells (Fig. 2A), *S.* Javiana invasion was significantly reduced in host cells pre-exposed to *L. acidophilus* or *L. rhamnosus* (Fig. 2B). Similarly, the cytotoxic effect of *S.* Javiana was significantly decreased in host cells pre-treated with *L. acidophilus*, *L. rhamnosus* and *L. casei* compared to cells infected with *S.* Javiana

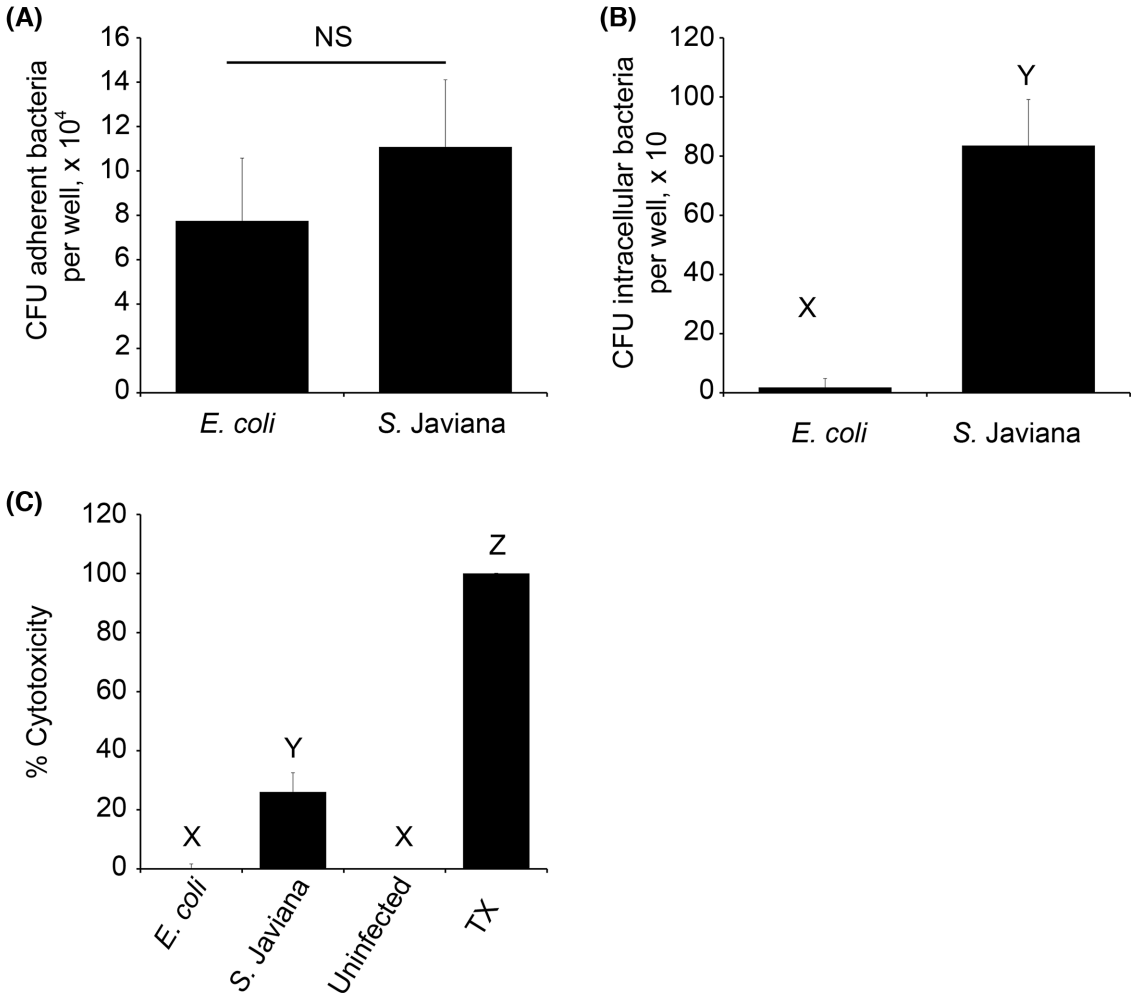

Figure 1. *Salmonella* Javiana adheres to, invades and exhibits cytotoxic effect on HT29-MTX intestinal epithelial cells. (A, B) HT29-MTX intestinal epithelial cells were inoculated with *E. coli* (non-pathogenic negative control) or S. Javiana, each at MOI 10. (A) At 1 h p.i., adherent bacteria were enumerated by plating. (B) At 2 h p.i., gentamicin-containing media (100 μg/ml) was added to the monolayers to kill extracellular bacteria, and 30 min later monolayers were washed and lysed with 0.1% Triton-X. Intracellular bacteria were enumerated from lysates by plating. (C) HT29-MTX intestinal epithelial cells were infected with *E. coli* (non-cytotoxic negative control) or S. Javiana. Uninfected cells served as a negative control, while 0.1% Triton-X (TX) was used as a positive control. At 2 h p.i., LDH was measured in HT29-MTX cell supernatants, and % cytotoxicity was determined as described in methods. In A and B, number of adherent or intracellular bacteria were compared between treatments. In C, % cytotoxicity was compared between treatments. NS indicates no significant difference in bacterial adhesion between treatments, while different letters (^{X,Y,Z}) indicate significant pairwise differences in invasion or cytotoxicity between treatment groups ($P < 0.05$).

alone (Fig. 2C). In contrast, pre-exposing HT29-MTX cells to non-pathogenic *E. coli* prior to S. Javiana infection had no impact on S. Javiana adhesion, invasion or cytotoxic effect (Fig. S3, Supporting Information). Data suggest that pre-exposing host cells to *L. acidophilus*, *L. rhamnosus* and *L. casei* has some protective effect that can reduce S. Javiana invasion and pathogen-induced host cell damage.

Heat-killed LAB do not affect S. Javiana invasion or S. Javiana-induced cytotoxicity

The mechanism by which probiotic bacteria might alter S. Javiana invasion and cytotoxic effect is unclear. Therefore, we performed similar invasion and cytotoxicity studies using live and HK-LAB, to ascertain whether LAB-mediated perturbation of S. Javiana infection is an active or passive probiotic process. While live LAB altered S. Javiana invasion and cytotoxic effect on host cells (Fig. 3A-B) there was no effect of HK *L. acidophilus* or *L. rhamnosus* on S. Javiana invasion (Fig. 3A). Similarly, there was no impact of HK *L. acidophilus*, *L. rhamnosus* or *L. casei* on S. Javiana-induced HT29-MTX cytotoxicity (Fig. 3B). Data indicate that perturbation of S. Javiana interaction with HT29-MTX cells is an active LAB process, and for that reason, all additional experiments were conducted with live LAB cultures.

LAB do not kill S. Javiana

We next sought to examine potential mechanisms by which LAB might reduce S. Javiana-induced cytotoxicity during HT29-MTX cell infection. Probiotic bacteria are well-known producers of bacteriocins that can kill enteric pathogens including *Salmonella* (Dobson *et al.* 2012). To determine whether the LAB used in our study exerted antimicrobial effects against S. Javiana, we grew S. Javiana alone or in co-culture with *L. acidophilus*, *L. rhamnosus*, *L. casei*, *L. plantarum* or *L. mesenteroides* and measured S. Javiana viability over time. There was no impact of any LAB species on S. Javiana viability during 6 h of co-culture (Fig. 4), suggesting that

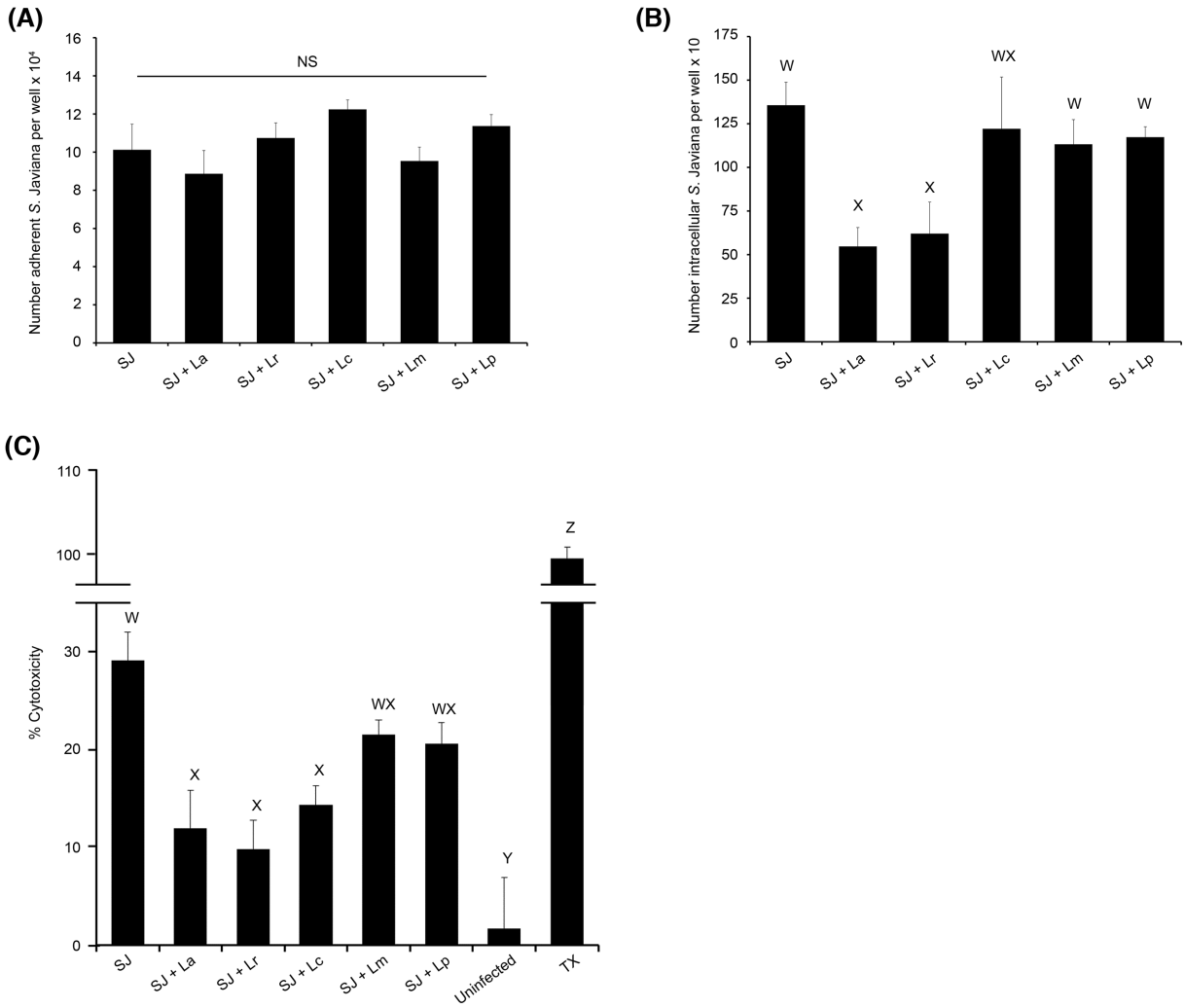

Figure 2. Pre-treating HT29-MTX cells with LAB prior to infection reduces S. Javiana invasion and S. Javiana-induced cytotoxicity. (A, B) HT29-MTX cells were infected with S. Javiana alone (MOI 10) or pre-treated for 1 h with individual LAB (MOE 10) prior to infection with S. Javiana. (A) At 1 h p.i., epithelial cells were washed and adherent S. Javiana were enumerated by plating. (B) At 2 h p.i., monolayers were treated with gentamicin (100 μg/ml) and 30 min later, epithelial cells were lysed and intracellular S. Javiana were enumerated by plating. (C) HT29-MTX cells were pre-treated with LAB (MOE 10) for 1 h prior to infection with S. Javiana (MOI 10) for 2 h. At 2 h p.i., LDH was measured in HT29-MTX cell supernatants and % cytotoxicity was determined. In A and B, number of adherent or intracellular S. Javiana were compared between treatments. In C, % cytotoxicity was compared between treatments. NS indicates no significant difference in S. Javiana adhesion between treatment groups, while different letters (^{W,X,Y,Z}) indicate significant pairwise differences in invasion or cytotoxicity ($P < 0.05$) between treatments. (SJ = S. Javiana, La = *L. acidophilus*, Lr = *L. rhamnosus*, Lc = *L. casei*, Lm = *L. mesenteroides*, Lp = *L. plantarum*, TX = Triton X-treated cells)

in our model, the LAB are not producing compounds that kill S. Javiana.

Exposure to LAB alters S. Javiana virulence gene expression during epithelial cell infection

Some strains of probiotics have been shown to exhibit anti-virulence effects on enteric pathogens such as *E. coli* and *Salmonella* (Medellin-Pena *et al.* 2007; Bayoumi and Griffiths 2012; Yang *et al.* 2014; Muyyarikkandy and Amalaradjou 2017; Peng *et al.* 2018), where exposure to the probiotic bacterium alters pathogen virulence gene expression. Given the fact that the LAB tested did not kill S. Javiana (Fig. 4), but did alter S. Javiana invasion (Fig. 2B) and its cytotoxic effect on host cells (Fig. 2C), we speculated that the LAB might be impacting expression of S. Javiana virulence traits. Therefore, we sought to examine the effect of *L. acidophilus*, *L. rhamnosus* and *L. casei* on expression of S. Javiana virulence genes *invA*, *prgH*, *pltA* and *cdtB*. The SPI-1 genes *invA* and *prgH* encode key parts of the SPI-1 type three secretion system (TTSS) that delivers effectors into intestinal epithelial cells, and have been shown in other NTS serovars to mediate epithelial invasion and cytotoxic effects on host cells (Galan and Curtiss 1989; Behlau and Miller 1993; Mills, Bajaj and Lee 1995; Collazo and Galan 1996). The *cdtB* gene also drives host cell invasion (Mezal, Bae and Khan 2014; Miller *et al.* 2018) and *cdtB* and *pltA* promote S. Javiana-induced host cell damage and cell cycle arrest (Mezal, Bae and Khan 2014; Miller and Wiedmann 2016; Miller *et al.* 2018). We assessed the impact of LAB on S. Javiana *invA*, *prgH*, *pltA* and *cdtB* expression in the presence of HT29-MTX cells to mimic conditions used in our previous infection assays and to account for the fact that *Salmonella* TTSS activity requires host cell contact (Galan and Collmer 1999). The HT29-MTX cells were pretreated with or without *L. acidophilus*, *L. rhamnosus* or *L. casei*, then infected with S. Javiana. At 2 h p.i., expression of S. Javiana *invA*, *prgH*, *pltA* and *cdtB* were assayed via qRT-PCR (Fig. 5). S. Javiana exposed to *L. acidophilus* and *L.*

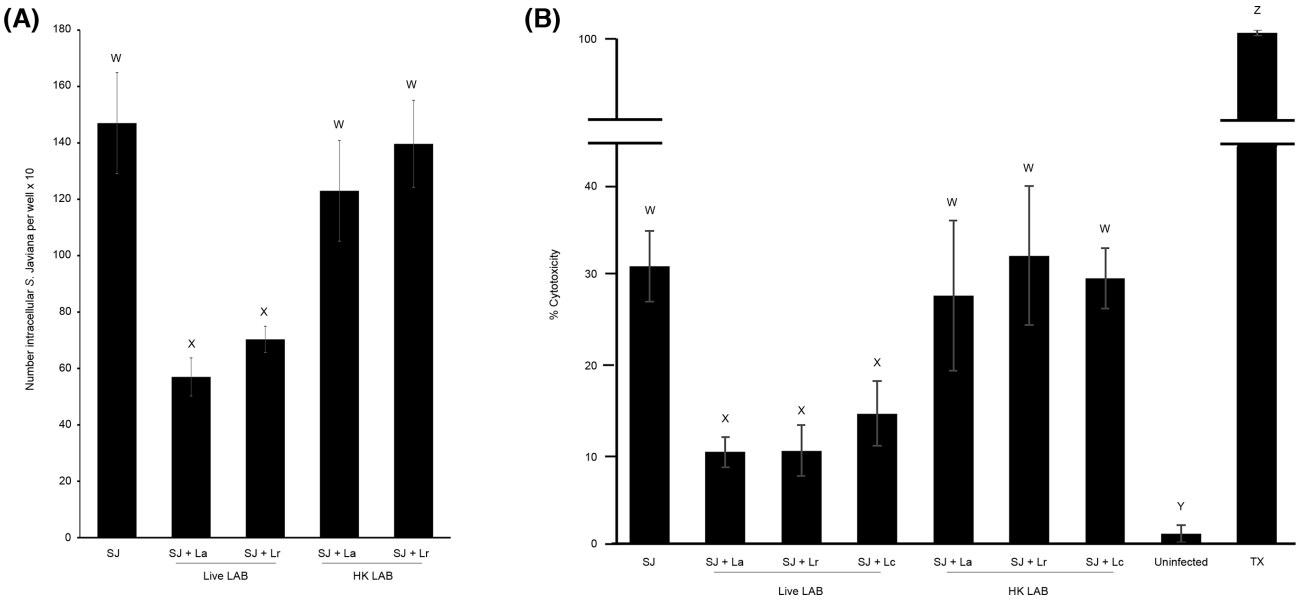

Figure 3. Heat-killed LAB do not alter S. Javiana invasion or S. Javiana-induced cytotoxicity. (A) HT29-MTX cells were infected with S. Javiana alone (MOI 10) or pre-treated for 1 h with live or heat-killed *L. acidophilus* or *L. rhamnosus* (MOE 10) prior to infection with S. Javiana. At 2 h p.i., monolayers were treated with gentamicin (100 μg/ml) and 30 min later, epithelial cells were lysed and intracellular S. Javiana were enumerated by plating. (B) HT29-MTX cells were either infected with S. Javiana alone (MOI 10) or pre-treated with live or heat-killed *L. acidophilus*, *L. rhamnosus* or *L. casei* (MOE 10) for 1 h prior to infection with S. Javiana. At 2 h p.i., LDH was measured in HT29-MTX cell supernatants and % cytotoxicity was determined. In A, the number of intracellular S. Javiana was compared between each treatment group, and in B the % cytotoxicity was compared between each treatment. Different letters (^{W,X,Y,Z}) indicate significant pairwise differences in intracellular bacteria or cytotoxicity ($P < 0.05$) between treatments. (SJ = S. Javiana, La = *L. acidophilus*, Lr = *L. rhamnosus*, Lc = *L. casei*, HK = heat-killed, TX = Triton X-treated cells).

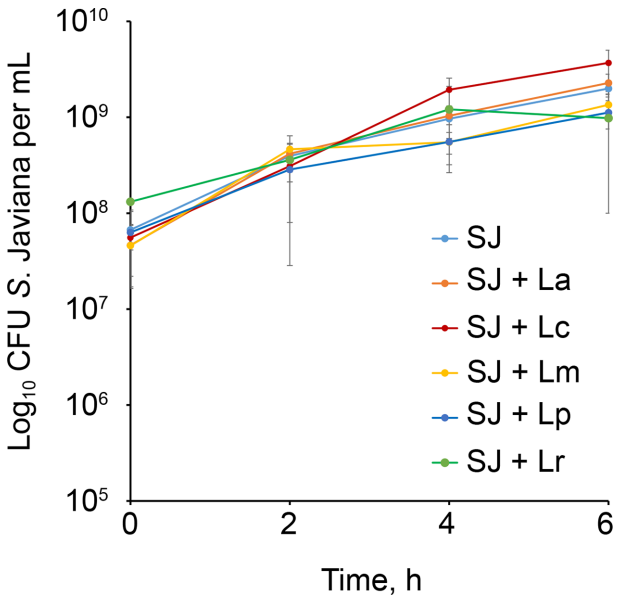

Figure 4. LAB do not kill S. Javiana. S. Javiana (SJ) was incubated in cell medium alone, or in presence of individual LAB strains. At 2, 4 and 6 h, viable S. Javiana were enumerated by plating. (SJ = S. Javiana, La = *L. acidophilus*, Lc = *L. casei*, Lm = *L. mesenteroides*, Lp = *L. plantarum*, Lr = *L. rhamnosus*).

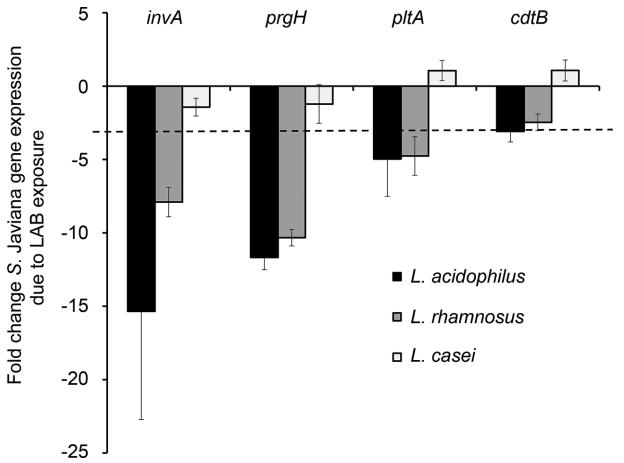

Figure 5. *L. acidophilus* and *L. rhamnosus* reduce S. Javiana virulence gene expression. HT29-MTX cells were pre-treated with or without LAB species *L. acidophilus*, *L. rhamnosus* or *L. casei* (MOE 10) for 1 h prior to infection with S. Javiana (MOI 10). At 2 h post-infection, bacteria were harvested, RNA was isolated and expression of S. Javiana *invA*, *prgH*, *pltA* and *cdtB* was analyzed via qRT-PCR. The effect of LAB exposure on S. Javiana gene expression was determined using the $\Delta\Delta C_T$ method. Data are presented as fold change in gene expression in S. Javiana cells exposed to LAB compared to S. Javiana alone. Genes exhibiting > 3-fold change (denoted by dotted line in figure) were considered altered for expression.

rhamnosus exhibited marked (8 to 15-fold) reduction in *invA* and *prgH* expression. In addition, S. Javiana exposed to *L. acidophilus* exhibited a 4.9-fold decrease in *pltA* and 3.1-fold reduction in *cdtB* expression, and S. Javiana exposed to *L. rhamnosus* exhibited a 4.8-fold reduction in *pltA* expression. We observed no effect of *L. casei* on expression of any S. Javiana virulence genes tested (Fig. 5). Together, data indicate that in our infection model, *L. acidophilus* and *L. rhamnosus* somehow cause a pronounced reduction in S. Javiana expression of key SPI-1 TTSS genes *invA* and *prgH*, and a moderate reduction in expression of expression of *pltA* and *cdtB*. Such effects on S. Javiana virulence expression might contribute to the anti-invasive and anti-cytotoxic effect of the LAB during S. Javiana infection, although additional studies are needed to confirm the role of *invA*, *prgH*, *pltA* and *cdtB* during S. Javiana infection of the HT29-MTX cell line.

LAB alter HT29-MTX cytokine production during S. Javiana infection

Having observed LAB-induced reduction in S. Javiana invasion, S. Javiana-mediated host cell cytotoxicity and virulence gene expression, we next examined whether LAB could alter the host inflammatory response by measuring the impact of LAB pre-treatment on HT29-MTX cytokine production during S. Javiana infection. HT29-MTX cells were either infected with S. Javiana alone or were pre-treated with *L. acidophilus, L. rhamnosus* or *L. casei* prior to infecting with S. Javiana for 6 h, after which infected cell supernatants were collected and assayed for inflammatory and anti-inflammatory cytokines using a commercial antibody array (Fig. 6A-D, Full array results in Table S1, Supporting Information). Pre-treating host cells with *L. acidophilus* and *L. casei* prior to infection significantly reduced epithelial production of inflammatory cytokines IL-6, TNFα and MCP-1 (Fig. 6A-C). In addition, while S. Javiana infection reduced host cell production of immunoregulatory cytokine IL-13, host cells exposed to *L. acidophilus* and *L. rhamnosus* prior to infection exhibited normal IL-13 production (Fig. 6D). We also measured the effect of LAB pre-exposure on S. Javiana intracellular viability at 6 h p.i., which is the same time-point at which cytokine production was assayed, and found fewer S. Javiana in host cells pre-treated with *L. acidophilus*, *L. rhamnosus* and *L. casei* than in untreated cells (Fig. 6E). Together, data point to a potential protective effect of LAB against potential host cell damage, inflammation, and pathogen intracellular survival during S. Javiana infection.

DISCUSSION

Probiotic microorganisms, and LAB in particular, have been shown to exhibit anti-infective properties against GI pathogens, including serovars of NTS (Burkholder and Bhunia 2009; Lievin-Le Moal and Servin 2014). Although precise mechanisms by which probiotic bacteria might limit infection remain inconclusive (Corr, Hill and Gahan 2009; Oelschlaeger 2010), proposed mechanisms are wide ranging and may include direct antimicrobial effects on pathogens (Alvarez-Sieiro *et al.* 2016), competition with pathogens for resources and colonization space within the host (Deriu *et al.* 2013; Lievin-Le Moal and Servin 2014), alteration of pathogen virulence (Medellin-Pena *et al.* 2007; Bayoumi and Griffiths 2010; Bayoumi and Griffiths 2012; Dutra *et al.* 2016) or modulation of host barriers and defenses (Anderson *et al.* 2010; Anderson *et al.* 2010). Moreover, the anti-pathogenic effects of probiotics are often specific to individual probiotic and pathogen strains (Sherman, Ossa and Johnson-Henry 2009; Campana, van Hemert and Baffone 2017). Despite the fact that S. Javiana is one of the leading NTS causes of foodborne gastroenteritis, the interaction of S. Javiana with host cells and the potential impact of LAB on S. Javiana pathogenesis was poorly understood. Using the human intestinal HT29-MTX epithelial cell line as an infection model, we report here that S. Javiana invades and has a cytotoxic effect on intestinal epithelial cells. We also show that three species of LAB—*L. acidophilus, L. rhamnosus* and *L. casei*—exhibit anti-pathogenic effects against S. Javiana in our infection model. Pre-treatment of host cells with *L. acidophilus* or *L. rhamnosus* reduced S. Javiana invasion, while *L. acidophilus, L. rhamnosus* and *L. casei* limited S. Javiana-induced cytotoxicity and intracellular survival. We also demonstrate reduced expression of S. Javiana virulence genes in the presence of *L. acidophilus* and *L. rhamnosus*, as well as an altered inflammatory response in host cells pre-treated with *L. acidophilus, L. rhamnosus* and *L. casei* prior to S. Javiana infection. Collectively, data suggest that *L. acidophilus*, *L. rhamnosus* and *L. casei* can exert a protective effect against S. Javiana infection, potentially by altering the virulence properties of the pathogen.

Our findings that *L. acidophilus* and *L. rhamnosus* reduced S. Javiana invasion are in agreement with previous reports which showed that LAB strains reduced invasion of other NTS serovars both *in vitro* (Tsai *et al.* 2005; Lin *et al.* 2008) and *in vivo* (Tsai *et al.* 2005; Lin *et al.* 2007; Chiu *et al.* 2008). These data also provide the first evidence of anti-invasive effects of LAB against S. Javiana. Similarly, we and others have shown that LAB can reduce host cell damage induced by enteric pathogens (Sherman *et al.* 2005), including NTS (Burkholder and Bhunia 2009; Eom, Song and Choi 2015). Although few studies have examined S. Javiana-induced host cell cytotoxicity, one recent study showed that while S. Javiana induced DNA damage and cell cycle arrest during infection of HIEC-6 human intestinal epithelial cells, the bacterium caused little host cell membrane damage or death (Miller *et al.* 2018). The discrepancies between their study and ours, in which we show a cytotoxic effect of S. Javiana—indicated by host cell release of the intracellular enzyme LDH—is potentially due to the use of different host cell lines. Here, our findings that S. Javiana-induced cytotoxicity was ameliorated in host cells pre-treated with *L. acidophilus, L. rhamnosus* and *L. casei* suggest a potential prophylactic effect of these LAB species against S. Javiana-mediated host cell damage.

Previous reports have shown that probiotics can interfere with infectious processes via active or passive mechanisms and, therefore, probiotic viability is not always a requirement for inhibition of infection or host immunomodulation (Sherman *et al.* 2005; Kataria *et al.* 2009). Some studies have demonstrated that killed preparations of probiotic strains can protect against pathogen binding (Hirano *et al.* 2017) or pathogen-induced epithelial damage (Popovic *et al.* 2019), and can alter the host response to pathogen challenge (Lopez *et al.* 2008; Popovic *et al.* 2019). In contrast, other reports have shown that probiotic viability is necessary for anti-infective properties. For example, Sherman et al (Sherman *et al.* 2005) showed that viable, but not HK, LAB prevented binding of enterohemorrhagic and enteropathogenic *E. coli* strains to T84 intestinal epithelial cells and prevented pathogen-induced alterations of epithelial barrier function. In addition, Roselli et al (Roselli *et al.* 2006) showed that live, but not HK, *Bifidobacterium animalis* and *L. rhamnosus* protected Caco-2 monolayers from inflammatory effects of enterotoxigenic *E. coli* infection. In this study, we demonstrate that only live LAB reduce S. Javiana invasion and cytotoxicity to host cells, as the anti-invasive effects of *L. acidophilus* and *L. rhamnosus* and the anti-cytotoxic effects of *L. acidophilus, L. rhamnosus* and *L. casei* were abolished when HT29-MTX cells were pre-exposed to HK LAB. This finding suggests that these LAB are somehow actively interfering with S. Javiana epithelial cell infection. The requirement for probiotic viability is likely specific for, and dependent on, the anti-infective mechanisms involved in individual probiotic-pathogen interactions, and the elucidation of mechanisms underlying the effects of LAB strains on S. Javiana epithelial infection will be the focus of future studies.

Several studies have reported that probiotic bacteria can exert anti-pathogenic effects by reducing pathogen virulence gene expression (Medellin-Pena *et al.* 2007; Bayoumi and Griffiths 2010; Bayoumi and Griffiths 2012; Yang *et al.* 2014; Younes *et al.* 2016; Muyyarikkandy and Amalaradjou 2017; Kiymaci *et al.* 2018; Zhao *et al.* 2018). Muyyarikkandy and Amalaradjou showed that the exposure of NTS serovars S. Typhimurium, S. Enteriditis and S. Heidelberg to LAB strains *L. rhamnosus, Lactobacillus bulgaricus* and *Lactobacillus paracasei* resulted in decreased pathogen

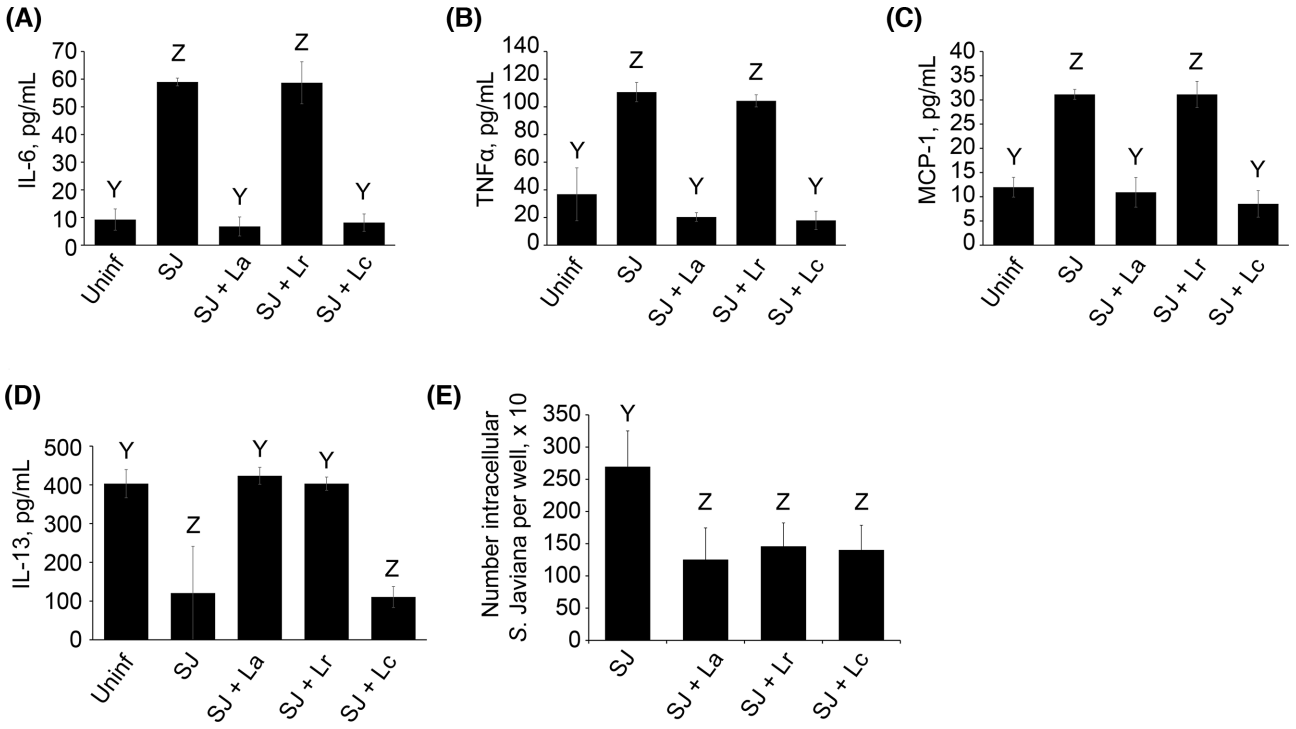

Figure 6. LAB alter host epithelial cytokine production during S. Javiana infection. (A-D) HT29-MTX cells were either uninfected or pre-treated with or without *L. rhamnosus*, *L. acidophilus* or *L. casei* (MOE 10) for 1 h prior to infection with S. Javiana (MOI 10). At 2 h p.i., gentamicin was added to cell media and monolayers were incubated for 4 h to allow for synthesis and secretion of cytokines. Infected cell supernatants were collected and analyzed for inflammatory and anti-inflammatory cytokines using a commercial antibody array (Ray Biotech). (E) To measure the number of intracellular S. Javiana present at 6 h p.i. (the time-point at which cytokine production was measured), HT29-MTX cells were either infected with S. Javiana alone or pre-treated with *L. acidophilus*, *L. rhamnosus* or *L. casei* for 1 h prior to S. Javiana infection. At 2 h p.i., gentamicin was added to the cell media and at 6 h p.i., epithelial cells were lysed and intracellular S. Javiana were enumerated by plating. In A-D, concentration of individual cytokines was compared between different treatments and in E, number of intracellular S. Javiana were compared between treatments. Different letters (^{Y,Z}) indicate a significant pairwise differences ($P < 0.05$) in cytokine concentration or S. Javiana intracellular survival between treatments. (Uninf = Uninfected, SJ = S. Javiana, La = *L. acidophilus*, Lr = *L. rhamnosus*, Lc = *L. casei*)

expression of motility genes, as well as SPI-1- and SPI-2-encoded genes for epithelial invasion, modulation of host actin cytoskeleton and evasion of macrophage intracellular defenses (Muyyarikkandy and Amalaradjou 2017). In that study, the LAB strains also impacted pathogen phenotype, decreasing NTS motility, invasion and intramacrophage survival. Another report demonstrated that individual and mixed LAB strains isolated from chicken intestinal contents decreased S. Typhimurium expression of SPI-1 virulence genes both *in vitro* and in an *in vivo* infection model, and the same LAB strains decreased S. Typhimurium extraintestinal translocation *in vivo* (Yang *et al.* 2014). Our finding that S. Javiana exposed to *L. acidophilus* and *L. rhamnosus* exhibited an 8–15-fold reduction in *invA* and *prgH* expression, and more moderate reductions in *pltA* and *cdtB* expression demonstrates that these LAB can modulate S. Javiana virulence. We speculate that LAB-mediated alteration of virulence expression could contribute to the anti-invasive and anti-cytotoxic effect of *L. acidophilus* and *L. rhamnosus* we observed in our invasion and cytotoxicity assays. However, these findings are a first step toward understanding how LAB may impact S. Javiana pathogenesis, and additional studies are needed to confirm the role of specific S. Javiana virulence genes, and the impact of LAB-mediated alterations in expression of those genes, during intestinal epithelial infection. In addition, it is possible that the influence of LAB on expression of specific virulence genes is the result of a broader effect of LAB on *Salmonella* virulence regulators (Bayoumi and Griffiths 2010; Muyyarikkandy and Amalaradjou 2017) such as *hilA*, the general regulator of SPI1 (Bajaj, Hwang and Lee 1995; Altier *et al.* 2000) and *ssrB*, the response regulator of SPI2 (Feng *et al.* 2004). Future studies will examine the impact of LAB strains and products on S. Javiana virulence regulation.

Probiotics can interfere with pathogen virulence expression in multiple ways that appear to be specific to individual probiotic and pathogen strains (Campana, van Hemert and Baffone 2017). For example, some probiotic bacteria secrete soluble compounds that interact with pathogen receptors (Medellin-Pena *et al.* 2007; Bayoumi and Griffiths 2012; Yang *et al.* 2014; Muyyarikkandy and Amalaradjou 2017), while others may require direct cell-to-cell contact with the pathogen to influence its virulence (Lievin-Le Moal and Servin 2014; Younes *et al.* 2016). In addition, recent studies have indicated that some LAB products alter virulence via disrupting pathogen quorum sensing signaling pathways (Li *et al.* 2011; Kiymaci *et al.* 2018; Zhao *et al.* 2018). Since our experiments were performed with a co-culture of LAB and S. Javiana in the context of host cell infection, we cannot ascertain whether the LAB signal to S. Javiana via a secreted compound or whether virulence inhibition requires direct probiotic-S. Javiana contact. Ongoing studies in our lab aim to characterize the nature of the LAB anti-virulence products and mechanisms by which such products alter S. Javiana virulence.

Although the finding that LAB can influence S. Javiana virulence gene expression is compelling, the underlying mechanisms by which LAB hinder S. Javiana invasion and host cell damage are likely complex and multifaceted. This is particularly true given the fact that *L. casei* pre-exposure reduced S. Javiana-induced cytotoxicity and intracellular survival, but had no effect

on expression of the virulence genes tested. It is possible that *L. casei* could alter expression of other *S.* Javiana virulence determinants not tested here, such as SPI-2-encoded genes that govern intracellular survival and pathogenesis, and such effects could manifest as decreased cytotoxic effect or reduced pathogen survival inside host cells. In addition, probiotics do exert biological effects on host cells as well as on pathogens, and such effects on the host can impact the outcome of infection (Lebeer, Vanderleyden and De Keersmaecker 2010). *L. casei, L.acidophilus* or *L. rhamnosus* could trigger defense processes in the host cell that contribute to reduced susceptibility to pathogen-induced damage and intracellular survival. Additional studies are warranted to elucidate the potentially distinct mechanisms by which these LAB species perturb the *S.* Javiana-host cell interaction.

Because probiotics form close associations with the intestinal epithelium, they can interact with host pattern recognition receptors (PRRs) and modulate inflammatory signaling (Lebeer, Vanderleyden and De Keersmaecker 2010; Kanmani and Kim 2018). Since NTS are known inducers of intestinal inflammation (Eckmann, Kagnoff and Fierer 1993; Jung *et al.* 1995; Eaves-Pyles *et al.* 2001; Lebeer, Vanderleyden and De Keersmaecker 2010), we examined the effect of *L. acidophilus, L. rhamnosus* and *L. casei* on HT29-MTX cytokine production in response to *S.* Javiana infection, and found that pre-treating host cells with *L. acidophilus* and *L. casei* significantly decreased production of inflammatory cytokines IL-6, TNFα and MCP-1, while *L. acidophilus* and *L. rhamnosus* prevented pathogen-induced reduction of anti-inflammatory IL-13. The effects of *L. acidophilus*, *L. rhamnosus* and *L. casei* on cytokine production by infected host cells correlate with our observation that *S.* Javiana intracellular viability at 6 h p.i. was reduced in host cells pre-exposed to those LAB strains. These data also concur with others who reported decreased inflammatory response in host cells and tissues pre-exposed to probiotic strains before infection with *S.* Typhimurium (Huang *et al.* 2015; Huang and Huang 2016; Yu *et al.* 2017), *S.* Infantis (Yang *et al.* 2017), and *Helicobacter pylori* (Lee *et al.* 2010; Yang *et al.* 2012), and indicate that *L. acidophilus, L. rhamnosus* and *L. casei* may elicit an immunomodulatory effect on host cells infected by *S.* Javiana.

Collectively, our findings demonstrate that *S.* Javiana exhibits an invasive and cytotoxic phenotype during infection of HT29-MTX intestinal epithelial cells, and that pre-exposing host cells to *L. acidophilus, L. rhamnosus* and *L. casei* can ameliorate *S.* Javiana virulence and alter the host cell inflammatory response. Since our experiments involved pre-treating host cells with LAB strains prior to infection, these data suggest a potential prophylactic effect of the LAB against *S.* Javiana infection. Indeed, others have shown that probiotics can have therapeutic, as well as prophylactic effects on infection (Lievin-Le Moal and Servin 2014). Additional studies are warranted to determine if these LAB strains could be effective if added at the same time or even after *S.* Javiana infection is initiated, to mimic a therapeutic model of probiotic use. Understanding how *S.* Javiana and host cells respond to LAB may inform future efforts to design functional probiotics to limit infection by this important NTS serovar.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSPD* online.

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Conflicts of interest. Authors declare No conflict of interests.

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