Converging effects of a *Bifidobacterium* and *Lactobacillus* probiotic strain on mouse intestinal physiology

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Lomasney KW, Cryan JF, Hyland NP. Converging effects of a *Bifidobacterium* and *Lactobacillus* probiotic strain on mouse intestinal physiology. Am J Physiol Gastrointest Liver Physiol 307: G241–G247, 2014. First published May 22, 2014; doi:10.1152/ajpgi.00401.2013.—Evidence has grown to support the efficacy of probiotics in the management of gastrointestinal disorders, many of which are associated with dysregulated fluid and electrolyte transport. A growing body of evidence now suggests that the host microbiota and probiotics can influence intestinal transport and that these effects often occur in a strain-dependent manner. In this study, we sought to investigate the effects of two therapeutically relevant organisms, *Bifidobacterium infantis* 35624 and *Lactobacillus salivarius* UCC118, on small intestinal transit, fecal output, and water content. Colon from mice fed both organisms displayed increased colonic TER, without a concomitant change in the gene expression of the tight junction proteins claudin 1 and occludin. However, *L. salivarius* UCC118 selectively inhibited neuronal evoked ion secretion in tissues from animals fed this particular probiotic. Consistent with this finding, the neurotoxin tetrodotoxin (TTx) significantly inhibited the short-circuit current response induced by TTx, although to a significantly lesser degree than the *L. salivarius* UCC118. Both strains similarly inhibited cholinergic-induced ion transport after addition to Ussing chambers. Taken together, these data suggest that *B. infantis* 35624 and *L. salivarius* UCC118 may be indicated in disorders associated with increased small intestinal transit, and, in particular for *L. salivarius* UCC118, neurally mediated diarrhea.

PROBIOTICS ARE DEFINED AS live microorganisms, that, when ingested in adequate amounts, provide health benefits to the host (9). *Lactobacilli* and *Bifidobacteria* represent two extensively studied probiotic species with demonstrated strain-dependent efficacy in a number of gastrointestinal diseases (31). In particular, *Bifidobacterium longum* subsp. *infantis* 35624 (*B. infantis* 35624) specifically relieves many of the symptoms observed clinically, in which *B. infantis* 35624 was more effective at improving IBS symptoms compared with a *Lactobacillus* strain or placebo (26). However, in the context of experimental colitis both *B. infantis* 35624 and *L. salivarius* UCC118 had similar effects (22). Therefore, these two commensal organisms appear to exert different effects on the host depending on the context in which they are studied. However, the effects of these two commensal organisms on host gastrointestinal physiology remain largely unknown.

In this regard, we sought to investigate the effects of *B. infantis* 35624 and *L. salivarius* UCC118 on intestinal transit and colonic ion transport. Intestinal fluid and electrolyte transport is a tightly regulated process that maintains a balance between electrolyte secretion and absorption (16). Electrogenic chloride ion secretion is driven by the apically expressed cystic fibrosis transmembrane conductance regulator (CFTR) and calcium-activated chloride channels, while epithelial sodium channels are responsible for active sodium absorption (1). Several studies now suggest that probiotics can directly or indirectly influence intestinal epithelial ion transport, and subsequently short-circuit current (*Isc*) (18). Specifically, *Lactobacillus acidophilus* can influence the activity of key regulators of intestinal ion transport including CFTR, the downregulated in adenoma (DRA) anion exchanger, and the basolaterally expressed sodium-potassium-chloride cotransporter (NKCC1) (4, 29, 30). These effects, however, are not unique to *Lactobacillus acidophilus*, and other strains also influence these targets, for example *Lactobacillus rhamnosus* (DRA) (29) and *Streptococcus thermophiles* (CFTR and NKCC1) (30). Moreover, the host microbiota (17), as well as specific probiotic and commensal organisms, have the capacity to alter secretagogue-evoked ion transport (6, 7, 11, 27, 30).

Studies examining the effects of *B. infantis* 35624 and *L. salivarius* UCC118 on intestinal ion transport, in the context of intestinal inflammation at least (8, 34), suggest that these two strains may differentially influence colonic secretomotor function. Therefore, in this study we sought to determine the effects of *B. infantis* 35624 and *L. salivarius* UCC118, in healthy animals, on small intestinal transit, fecal output, and fecal water content. We also examined the effects of both strains on colonic ion transport and secretomotor function after either 2-wk feeding or following acute addition to Ussing chambers.

MATERIALS AND METHODS

All drugs were obtained from Sigma-Aldrich (Ireland) unless otherwise stated. The following compounds were used, with the final concentration and diluent in parenthesis: amiloride (100 μM dissolved in distilled water dH2O), betahanechol (100 μM; dissolved in dH2O),...
forskolin (10 μM dissolved in DMSO), furosemide (100 μM dissolved in DMSO), tetrodotoxin (300 nM dissolved in sodium citrate buffer), and veratridine (30 μM; dissolved in 70% ethanol). Krebs buffer was prepared as follows: 1.2 mM NaH2PO4, 117 mM NaCl, 4.8 mM KCl, 1.2 mM MgCl2, 25 mM NaHCO3, 11 mM CaCl2, and 10 mM glucose. Chloride-free buffer consisted of 116 mM NaNO3, 5.4 mM KNO3, 1.2 mM NaH2PO4, 2.5 mM Ca(NO3)2, 1.2 mM Mg(NO3)2, 22 mM NaHCO3, and 11.2 mM glucose.

Animals

Male Swiss Webster mice (22–35 g) were obtained from Harlan UK. All animals were kept on a 12:12-h dark-light cycle (lights on at 7 AM) with food and water ad libitum. Animals were fasted overnight prior to measurement of small intestinal transit. All mouse experiments were conducted following institutional ethics guidelines and were in full accordance with the European Community Directive (86/609/EEC), and approved by the Animal Experimentation Ethics Committee of University College Cork.

Preparation of Probiotics

Freeze-dried preparations of B. infantis 35624 and L. salivarius UCC118 were obtained from Alimentary Health (Cork, Ireland) and prepared as previously described (23). Briefly, both B. infantis 35624 and L. salivarius UCC118 were produced in large-scale fermenters, the bacterial pellet was harvested and washed, and the resultant supernatant was discarded. The biomass was subsequently freeze-dried with cryoprotectant. A proprietary cryoprotectant (Alimentary Health) was used as vehicle for comparison throughout. Colony-forming units (CFU) were determined by the spread plate technique.

Probiotic feeding. Freeze-dried probiotics and vehicle were reconstituted in deionized water, and a volume of 300 μl containing 1 × 106 CFU/ml was administered to the mice daily by oral gavage for 2 wk. Mice in the same treatment groups were housed in pairs.

Preparation of probiotics for Ussing chamber studies. For Ussing chamber studies bacteria were resuspended in Krebs buffer and added to the mucosal reservoir of the Ussing chamber to yield 1 × 106 CFU/ml. The pH of the final suspensions was assessed: Krebs buffer (pH 7.0), vehicle + Krebs buffer (pH 7.01). B. infantis 35624 + Krebs buffer (pH 6.94), and L. salivarius UCC118 + Krebs buffer (pH 6.97). To heat kill the probiotics, suspensions were maintained at 100°C for 30 min and were subsequently allowed to cool for 15 min prior to addition to the Ussing chamber. Probiotics were determined to be viable after resuspension in Krebs buffer and failed to grow following heat treatment (data not shown).

Measurement of Small Intestinal Transit

To examine small intestinal transit, mice fed B. infantis 35624, L. salivarius UCC118 or vehicle for 2 wk were administered activated charcoal (0.5 g in 10 ml + 0.5% methylcellulose) by oral gavage (0.1 ml/10 g). Twenty minutes later mice were euthanized by cervical dislocation, the distal colon was removed and placed in chilled Krebs solution. Seromuscular stripping was carried out by blunt dissection under a stereomicroscope, and both the longitudinal and circular muscle layers were removed. The resulting mucosal-submucosal segments were mounted in Ussing chambers (exposed tissue area of 0.12 cm²), maintained in Krebs solution at 37°C and oxygenated with carbogen gas (95% O2, 5% CO2). Tissues were voltage clamped to zero using an automatic voltage clamp (DVC-1000/EVC-4000, World Precision Instruments, Sarasota, FL). Once a stable baseline was achieved, basal Isc and transepithelial resistance (TER) were recorded. Tissue responses to the neural activator, veratridine, as well as to bethanechol and forskolin, to assess calcium- and cAMP-mediated ion secretion, respectively, were then measured. All measurements were continuously recorded on a computer using LabTrax data acquisition hardware and analyzed using DataTrax software (World Precision Instruments).

Using a separate group of experimentally naïve mice, we assessed the acute effects of B. infantis 35624 or L. salivarius UCC118 on colonic ion transport. Mice were euthanized and colonic tissue was prepared as above. Once a stable baseline was achieved, basal Isc and TER were recorded, and either B. infantis 35624, L. salivarius UCC118, or vehicle was added to the mucosal reservoir. The resultant peak change in Isc was recorded, after which bethanechol (15 min) and forskolin (15 min) were routinely added to the serosal reservoir. To investigate the contribution of chloride ions to the change in Isc induced by B. infantis 35624 or L. salivarius UCC118, Krebs was replaced with chloride-free Krebs buffer. To examine the contribution of epithelial sodium channel (ENaC), NKCC1, and the enteric nervous system on probiotic-stimulated changes in Isc, tissues were pretreated with amiloride (mucosal), furosemide (serosal), or TTX (serosal), respectively.

Analysis of Tight Junction Protein Gene Expression

Total RNA was extracted by use of a commercially available kit (Qiagen, Valencia, CA). mRNA was reverse transcribed by using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) in a G-Storm thermocycler (G-Storm, Ringmer, East Sussex, UK). Gene expression was analyzed by qualitative real-time polymerase chain reaction using TaqMan Gene expression assays and the AB7300 system (Applied Biosystems). Occludin (Ocln) was detected with the probe no. Mm00500912_m1 and claudin 1 (Clcn1) with probe no. Mm0516701_ml. The expression of each gene was normalized to β-actin. All samples were analyzed in duplicate.

Statistics

Data were analyzed and graphed with GraphPad Prism 5 by either one- or two-way ANOVA followed by post hoc analysis using Bonferroni’s multiple comparison test. Data are presented as means ± SE and a P < 0.05 was considered statistically significant.

RESULTS

Effect of B. infantis 35624 or L. salivarius UCC118 on Small Intestinal Transit, Fecal Output, and Fecal Water Content After Probiotic Feeding

Clinical evidence suggests that B. infantis 35624 may differentially influence bowel movement difficulty in IBS patients, without a concomitant change in stool consistency, relative to a Lactobacillus strain (26). Therefore, we assessed whether B. infantis 35624 or L. salivarius UCC118 differentially influenced small intestinal transit as well as fecal output and water content in probiotic fed mice. Both B. infantis 35624...
and *L. salivarius* UCC118 significantly decreased small intestinal transit relative to vehicle after 2 wk of feeding (Fig. 1; *P* < 0.05). However, neither strain altered stress-induced fecal output or water content (Fig. 2, A and B). Therefore, both strains exert similar inhibitory effects on small intestinal transit but do not appear to influence fecal output or water content.

**Effect of *B. infantis* 35624 or *L. salivarius* UCC118 on Transepithelial Resistance and Tight Junction Protein Gene Expression After Probiotic Feeding**

Probiotics have the capacity to influence intestinal epithelial barrier function (28). In particular, *L. salivarius* UCC118 has demonstrated beneficial effects on colonic permeability during colitis (8, 24) and can protect against hydrogen peroxide-induced reductions in TER in vitro (24). Therefore, after feeding mice *B. infantis* 35624 or *L. salivarius* UCC118 for 2 wk, we assessed colonic TER in Ussing chambers and analyzed tissues for the expression of two tight junction protein genes known to be influenced by *L. salivarius* UCC118, namely claudin 1 and occludin (24). Both *B. infantis* 35624 and *L. salivarius* UCC118 increased TER, the latter significantly so (*P* < 0.05, Fig. 3). However, neither probiotic altered the gene expression of either claudin 1 or occludin (Table 1). The observed increase in TER may be indicative of potential barrier-enhancing properties of the probiotic strains.

**Ex vivo Assessment of Baseline and Secretagogue-Evoked Colonic Ion Transport in Animals Fed *B. infantis* 35624 or *L. salivarius* UCC118**

To further investigate the effects of *B. infantis* 35624 or *L. salivarius* UCC118 on colonic physiology we examined tissue responses to a number of secretory stimuli in animals fed both bacterial strains. Specifically, we examined calcium-stimulated ion transport using the cholinomimetic bethanechol, cAMP-mediated ion transport using forskolin, and neurally mediated ion transport using the sodium channel activator veratridine. Both strains reduced baseline *I*~sc~ relative to vehicle (*P* < 0.05, Fig. 4A); however, neither strain significantly influenced the tissue response to either bethanechol (Fig. 4B) or forskolin (Fig. 4C). Veratridine-induced ion transport was, however, significantly decreased in tissues collected from animals fed *L. salivarius* UCC118 (Fig. 4D; *P* < 0.05). Thus both *B. infantis* 35624 and *L. salivarius* UCC118 similarly influence basal colonic ion transport. However, *L. salivarius* UCC118 selectively inhibits neurally mediated responses.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Vehicle (n = 8)</th>
<th><em>B. infantis</em> (n = 11)</th>
<th><em>L. salivarius</em> (n = 9)</th>
</tr>
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<tbody>
<tr>
<td>Occludin</td>
<td>0.88 ± 0.15</td>
<td>0.85 ± 0.13</td>
<td>0.99 ± 0.15</td>
</tr>
<tr>
<td>Claudin 1</td>
<td>0.48 ± 0.11</td>
<td>0.43 ± 0.07</td>
<td>0.47 ± 0.08</td>
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Table 1. Colonic gene expression of the tight junction proteins occludin and claudin 1 in mouse colon following probiotic feeding with *Bifidobacterium infantis* 35624 and *Lactobacillus salivarius* UCC118

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**Fig. 1.** Small intestinal transit was significantly decreased in animals fed *Bifidobacterium infantis* 35624 or *Lactobacillus salivarius* UCC118 relative to vehicle in vivo. *P* < 0.05; *n* = 7–8.

**Fig. 2.** Neither *Bifidobacterium infantis* 35624 nor *Lactobacillus salivarius* UCC118 significantly influenced stress-induced fecal pellet output (A) or water content (B) in response to a mild stress; *n* = 10–11.

**Fig. 3.** Colonic transepithelial resistance (TER) was assessed in mucosa-submucosa preparations mounted in Ussing chambers. Tissues from animals fed *Bifidobacterium infantis* 35624 displayed a significantly increased TER relative to those from the vehicle-fed group. *P* < 0.05; *n* = 8–9.
Characterization of the Acute Effects of B. infantis 35624 or L. salivarius UCC118 on Colonic Ion Transport in Ussing Chambers

To characterize the potential mechanisms underlying the effects of B. infantis 35624 and L. salivarius UCC118 on colonic ion transport, we took a number of experimental approaches. These included 1) ion exclusion studies using chloride-free Krebs to investigate the contribution of chloride ions to the \( I_{sc} \) response evoked by B. infantis 35624 or L. salivarius UCC118; 2) pharmacological studies using furosemide, to inhibit basolateral NKCC1, and amiloride, to inhibit epithelial sodium channels; and 3) neural inhibition using the neurotoxin TTx to examine the contribution of the enteric nervous system to the \( I_{sc} \) response elicited by B. infantis 35624 or L. salivarius UCC118. In additional experiments, the bacterial strains were heat killed to investigate whether viable bacteria were required to induce their effects on colonic ion transport. The acute effects of B. infantis 35624 or L. salivarius UCC118 on bethanechol- and forskolin-induced changes in \( I_{sc} \) were also examined.

Both viable and heat-killed B. infantis 35624 and L. salivarius UCC118 significantly increased baseline \( I_{sc} \) relative to vehicle following mucosal addition to the Ussing chamber (Fig. 5). In experiments conducted with chloride-free Krebs, the response to L. salivarius UCC118 was significantly increased relative to vehicle (Table 2). Neither basolateral addition of furosemide nor apical addition of amiloride significantly altered the \( I_{sc} \) response elicited by either bacterial strain (Table 2). Pretreatment of tissues with TTxs significantly inhibited the response to both B. infantis 35624 (\( P < 0.01 \); Fig. 6A) and to L. salivarius UCC118 (\( P < 0.001 \); Fig. 6B), with the extent of inhibition greater for L. salivarius UCC118 (~60% reduction in \( I_{sc} \) response; Fig. 6, A and B). Both B. infantis 35624 (\( P < 0.05 \) ) and L. salivarius UCC118 (\( P < 0.01 \) ) significantly inhibited bethanechol-induced \( I_{sc} \) (Fig. 7A), and neither strain influenced cAMP-mediated ion transport (Fig. 7B). Thus chloride ions do not appear to significantly contribute to the increase in \( I_{sc} \) observed in response to acute exposure of tissues to B. infantis 35624 or L. salivarius UCC118, although their removal influences the response to L. salivarius UCC118. Both strains interact with the enteric nervous system, with L. salivarius UCC118 displaying greater sensitivity to the neurotoxin and both bacteria similarly inhibit cholinergic-induced ion transport.

DISCUSSION

We have demonstrated that both B. infantis 35624 and L. salivarius UCC118 similarly inhibit small intestinal transit, increase TER, and decrease baseline \( I_{sc} \) in tissues obtained from mice fed each strain. However, in animals fed L. salivarius UCC118, neurally evoked colonic ion transport was selectively inhibited. The TTx sensitivity of the L. salivarius

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**Fig. 4.** Baseline (A) and stimulated (B–D) short-circuit current (\( I_{sc} \)) responses were assessed in colonic tissues from animals fed Bifidobacterium infantis 35624 or Lactobacillus salivarius UCC118. Both bacteria significantly decreased basal \( I_{sc} \) (A), and neither had a significant effect on either bethanechol- or forskolin-induced ion transport (B and C). Lactobacillus salivarius UCC118 significantly inhibited veratridine-induced ion transport relative to both vehicle and Bifidobacterium infantis 35624 (D). *\( P < 0.05 \), vehicle vs. probiotic; #\( P < 0.05 \), Bifidobacterium infantis 35624 vs. Lactobacillus salivarius UCC118; \( n = 5–8 \).

**Fig. 5.** Acute effects of Bifidobacterium infantis 35624 and Lactobacillus salivarius UCC118 on colonic \( I_{sc} \) were measured following addition of either microbe to the mucosal compartment of the Ussing chamber. Both viable and heat-killed Bifidobacterium infantis 35624 (A) and Lactobacillus salivarius UCC118 (B) significantly increased baseline \( I_{sc} \) relative to vehicle. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \); \( n = 5–9 \).
Table 2. The effect of chloride ion exclusion and inhibition of ENaC and NKCC1 on the Isc response elicited by Bifidobacterium infantis 35624 and Lactobacillus salivarius UCC118 in mouse colon

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>B. infantis 35624</th>
<th>L. salivarius UCC118</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krebs</td>
<td>18.91 ± 4.21 μA·cm⁻² (n = 6)</td>
<td>22.53 ± 7.45 μA·cm⁻² (n = 6)</td>
</tr>
<tr>
<td>Chloride-free Krebs</td>
<td>17.30 ± 3.24 μA·cm⁻² (n = 6)</td>
<td>52.70 ± 12.00 μA·cm⁻² (n = 6)</td>
</tr>
<tr>
<td>Vehicle (dH₂O)</td>
<td>20.74 ± 5.18 μA·cm⁻² (n = 6)</td>
<td>25.52 ± 3.32 μA·cm⁻² (n = 5)</td>
</tr>
<tr>
<td>Amiloride</td>
<td>26.07 ± 1.31 μA·cm⁻² (n = 10)</td>
<td>27.47 ± 4.47 μA·cm⁻² (n = 5)</td>
</tr>
<tr>
<td>Vehicle (DMSO)</td>
<td>25.24 ± 3.71 μA·cm⁻² (n = 6)</td>
<td>28.81 ± 0.71 μA·cm⁻² (n = 6)</td>
</tr>
<tr>
<td>Furosemide</td>
<td>21.77 ± 7.94 μA·cm⁻² (n = 7)</td>
<td>23.12 ± 3.04 μA·cm⁻² (n = 6)</td>
</tr>
</tbody>
</table>

ENaC, epithelial sodium channel; Isc, short-circuit current; dH₂O, distilled water; DMSO, dimethyl sulfoxide. *P < 0.05, Krebs vs. chloride-free Krebs.

UCC118 response observed in Ussing chamber studies further supports a predominantly neural effect of this organism on secretomotor function. Also notable, following acute exposure of tissues to either B. infantis 35624 or L. salivarius UCC118 in Ussing chambers, was the similar inhibition of cholinergic-induced ion transport by both microbes. Additional experiments revealed that removal of chloride ions significantly increased the magnitude of the baseline response to L. salivarius UCC118 only and that both strains induced similar effects on baseline Isc irrespective of heat-killing in acute tissue experiments.

Recent evidence suggests that probiotics exert both strain- and region-specific effects on gut physiology (38). For example, Lactobacillus rhamnosus and Lactobacillus reuteri differentially influence migrating myoelectric complex parameters in mouse jejunal and colonic preparations (38). Moreover, the probiotic mix VSL#3 exerts region-specific effects on gastrointestinal motility in isolated tissue preparations (21). Our data suggest that the inhibitory effects of B. infantis 35624 and L. salivarius UCC118 on small intestinal transit are not strain dependent and do not influence fecal output or water content when examined under mildly stressful conditions. The mechanisms underlying such effects induced by B. infantis 35624 and L. salivarius UCC118 on small intestinal transit are currently unclear. However, evidence suggests that probiotics may influence motility at the level of the enteric nervous system (15, 35, 36) or through direct effects on smooth muscle cells (5). Clinically, increased small bowel transit has been associated with diarrhea-predominant IBS, stress, and anxiety (12). Therefore, probiotic interventions, using bacterial strains with the capacity to slow small intestinal transit, may be particularly efficacious in these contexts.

It has been suggested that an altered microbiota may contribute to intestinal inflammation, and therefore disrupted epithelial barrier function (25). Several probiotics exert beneficial effects on epithelial barrier function by regulating, for example, mucin gene expression, production of β-defensins, bacte- riocins, and secretory IgA as well as modulation of tight

![Image](http://ajpgi.physiology.org/)

Fig. 6. To investigate the sensitivity of the Bifidobacterium infantis 35624- and Lactobacillus salivarius UCC118-induced effects on Isc to neural blockade, tissues were pretreated with tetrodotoxin (TTx) prior to luminal addition of the microbes. Both Lactobacillus salivarius UCC118 and Bifidobacterium infantis 35624 displayed sensitivity to TTx (A). Representative trace demonstrating the effect of TTx on the Lactobacillus salivarius UCC118-induced response in mouse colon (B). **P < 0.01, ***P < 0.001, Bifidobacterium infantis 35624 vs. Lactobacillus salivarius UCC118; #P < 0.05, ###P < 0.001, vehicle vs. TTx; n = 5–6.

![Image](http://ajpgi.physiology.org/)

Fig. 7. Acute effects of Bifidobacterium infantis 35624 or Lactobacillus salivarius UCC118 on betahaloehol- and forskolin-induced ion transport were assessed following mucosal addition of the microbes to the Ussing chamber. Both Bifidobacterium infantis 35624 and Lactobacillus salivarius UCC118 significantly decreased betahaloehol-induced ion transport (A), whereas neither bacteria had an effect on forskolin responses (B). **P < 0.01, ***P < 0.001; n = 4–6.
junction proteins (28). We observed an increase in colonic TER in tissues from animals fed \textit{B. infantis} 35624 or \textit{L. salivarius} UCC118 for 2 wk, which suggests that both strains may have the capacity to increase epithelial barrier function. However, we did not find an associated change in the gene expression of two tight junction genes, claudin 1 and occludin. In vivo studies, in the context of experimental colitis, support a beneficial effect of \textit{L. salivarius} UCC118 on intestinal permeability and suggest that this effect is bacteriocin dependent, since bacteriocin-negative \textit{L. salivarius} UCC118 did not exert the same effect in vivo (24). Moreover, in vitro studies further confirmed the beneficial effect of \textit{L. salivarius} UCC118 on hydrogen peroxide-induced changes in both TER and permeability, and this beneficial effect was accompanied by a redistribution of tight junction proteins, including claudin 1 and occludin (24). The later findings may account for the absence of any change in gene expression observed in our study. However, changes in TER may not necessarily accompany changes in permeability. For instance, the beneficial effect of \textit{L. salivarius} UCC118 on colitis-associated deficits in permeability were not accompanied by a change in TER or $I_{sc}$ (8). Our study, however, is the first to indicate a role for \textit{B. infantis} 35624 in the modulation of TER. Potential mechanisms underlying this effect may include changes in tight junction gene expression other than claudin 1 and occludin, localization, or protein kinase activity previously described for other strains of \textit{B. infantis} (3, 7).

There is now a growing appreciation that one of the mechanisms by which microbes interact with the host is through bacterial signaling to the enteric nervous system (32). With respect to secretomotor function, our data indicate that \textit{L. salivarius} UCC118 preferentially inhibits neurally driven responses following chronic administration. Acute exposure of colonic preparations to \textit{L. salivarius} UCC118 in the presence of TTx further suggests a role for the enteric nervous system in mediating the effects this particular microbe on colonic ion transport. The enteric nervous system also appears, although to a lesser degree, to contribute to the effects of \textit{B. infantis} 35624 on the $I_{sc}$ response elicited after short-term exposure in vitro. It is known, however, that different microbes have the capacity to differentially influence enteric nerve activity (2, 14, 15, 20). For example, \textit{Lactobacillus reuteri} (15) and \textit{Bacteroides fragilis} (20) enhance the activity of, or activate, enteric neurons whereas \textit{Bifidobacterium longum} exerts an inhibitory effect (2, 14). The inhibitory effect of \textit{L. salivarius} UCC118, in particular, on enteric nerve activity may be of therapeutic importance in pathogen-mediated diarrheal diseases, particularly those caused by \textit{Salmonella} spp., \textit{Clostridium difficile} (13), and rotavirus (19), in which neural pathways have been implicated.

The inhibition of cholinergic-induced ion transport by \textit{L. salivarius} UCC118 and \textit{B. infantis} 35624, in acute Ussing chamber studies, provides evidence of their antisecretory properties and has previously been observed for other probiotic strains, notably, \textit{Bifidobacterium breve} C50 (11). In contrast, \textit{Bifidobacterium breve} 15698, \textit{Lactobacillus rhamnosus} 10893, and \textit{Escherichia coli} L15 had no such effect (11). In the same study, strain dependency was also noted with respect to the effects of the different bacterial strains on forskolin-induced ion transport (11). However, we observed no effect of either \textit{B. infantis} 35624 or \textit{L. salivarius} UCC118 on forskolin-induced responses. Therefore, both probiotics appear to selectively inhibit cholinergic-induced ion transport relative to cAMP-mediated responses and do not display strain selectivity in this regard.

On the basis of the TTx sensitivity of the response elicited by \textit{B. infantis} 35624 or \textit{L. salivarius} UCC118 in Ussing chamber studies, we speculate that a neural reflex may underlie the relatively immediate change in $I_{sc}$ we observed; however, whether the increase in $I_{sc}$ is secretory or absorptive in nature remains to be fully elucidated. Removal of chloride ions significantly increased the tissue response to \textit{L. salivarius} UCC118. In the absence of chloride ions, increased secretion of bicarbonate could account for an increase in $I_{sc}$, similar to that we observed in response to \textit{L. salivarius} UCC118. However, $Cl^{-}/HCO_{3}^{-}$ exchange, facilitated by apically expressed DRA, for example, is dependent on the presence of chloride ions, the activity of which would likely be reduced in the absence of chloride. Nonetheless, bicarbonate secretion may also occur via CFTR (10). Moreover, in the absence of external chloride, the permeability of CFTR to bicarbonate may increase (33). Further characterization of the ionic nature of the response elicited by both \textit{B. infantis} 35624 and \textit{L. salivarius} UCC118 is now warranted to better understand the ion transport processes underlying their effects on $I_{sc}$. Currently, we can only speculate on the divergent effects of \textit{B. infantis} 35624 and \textit{L. salivarius} UCC118 on secretory responses observed between tissues collected from animals fed both strains and those exposed to the probiotics acutely. Evidence, however, suggests that several microbes have the capacity to alter the expression of key ion channels or exchangers, which may require a greater period of exposure than those we examined in our Ussing chamber studies (18). Therefore, we hypothesize that the length of time to which the colonic tissues were exposed to the probiotics in Ussing chambers may have been insufficient for changes in the trafficking or expression of ion channels, or for the effects of the probiotics on the local immune system, and subsequent indirect influence on $I_{sc}$ to occur.

Collectively, our data suggest that both \textit{B. infantis} 35624 and \textit{L. salivarius} UCC118 significantly affect gastrointestinal physiology; slowing small intestinal transit and significantly influencing colonic secretomotor function. Both display antisecretory effects following addition to Ussing chambers, inhibiting cholinergic-induced responses. Moreover, \textit{L. salivarius} UCC118 preferentially inhibits neurally evoked ion transport after probiotic feeding, suggesting that this strain, in particular, may be of benefit in countering diarrhea associated with neural activation. Further studies are now justified with respect to investigating the effects of \textit{B. infantis} 35624 and \textit{L. salivarius} UCC118 in disease contexts associated with increased small intestinal transit and neurally mediated diarrhea in particular.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS
K.W.L. performed experiments; K.W.L. and N.P.H. analyzed data; K.W.L. and N.P.H. interpreted results of experiments; K.W.L. prepared figures; K.W.L., J.F.C., and N.P.H. drafted manuscript; K.W.L., J.F.C., and N.P.H. edited and revised manuscript; J.F.C. and N.P.H. conception and design of research; J.F.C. and N.P.H. approved final version of manuscript.

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