

Effects of probiotic bacteria on gastrointestinal motility in guinea-pig isolated tissue

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Abstract

AIM: To evaluate the intestinal motility changes evoked by 8 bacterial strains belonging to *Bifidobacterium*, *Lactobacillus* and *Streptococcus* genera within the probiotic preparation VSL#3.

METHODS: Ileum and proximal colon segments isolated from guinea-pigs were used as a study model. Entire cells and cell fractions (cell debris, cell wall fraction, cytoplasmatic fraction, proteinaceous and non-proteinaceous cytoplasmatic components) of VSL#3 strains and, as controls, *Escherichia coli*, *Salmonella aboni* and *Bacillus licheniformis* were tested in this *in vitro* model.

RESULTS: Among the bacterial cell fractions tested, only the cytoplasmatic fraction modified intestinal motility. *Lactobacillus* strains stimulated the contraction of ileum segment, whereas all probiotic strains tested induced proximal colon relaxation response. The non-proteinaceous cytoplasmatic components were responsible for the colon relaxation.

CONCLUSION: The results obtained in this study suggest that the proximal colon relaxation activity showed by the probiotic bacteria could be one of the possible mechanisms of action by which probiotics exert their positive effects in regulating intestinal motility.

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Key words: Probiotics; Intestinal motility; Guinea-pigs; Ileum; Colon

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INTRODUCTION

Probiotics are viable microbial cells that upon ingestion in specific numbers appear to have beneficial effects on the health and well-being of the host, beyond inherent basic nutrition^[1]. These health-promoting effects are predominantly related to reinforcement of the intestinal mucosal barrier against enteropathogens' colonization, immunostimulation and immunomodulation, anticarcinogenic and antimutagenic activities, improvement of lactose utilization, and reduction of serum cholesterol^[2].

Most probiotics are bacteria members of the genera *Lactobacillus* and *Bifidobacterium*, which represent important components of human gastrointestinal flora. However, other nonpathogenic bacteria, such as *Streptococcus*, some strains of *Escherichia coli* and *Enterococcus faecium*, and yeasts, such as *Saccharomyces boulardii*, have been used in probiotic preparations^[3].

Experimental and clinical studies support the use of probiotics in the treatment of intestinal disorders such as infectious diarrhea^[4-7], antibiotic diarrhea^[8-10], traveller's diarrhea^[11-13], irritable bowel syndrome or functional diarrhea^[14-18] and inflammatory bowel disease^[19-23]. In recent double-blind placebo controlled trials the efficacy of the probiotic preparation VSL#3 has been shown as maintenance treatment and prophylactic therapy in patients with diarrhea-predominant irritable bowel syndrome and pouchitis^[18,22-24]. VSL#3 (VSL Pharmaceuticals, Ft. Lauderdale, FL, USA) contains a mixture of eight different bacterial species at very high concentrations (450 billions/sachet of viable lyophilized bacteria). The preparation consists of three strains of *bifidobacteria* (*Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*), four strains of *lactobacilli* (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*) and one strain of *Streptococcus thermophilus*.

To the best of our knowledge, there are no experimental data evaluating the effects of probiotic bacteria on

the intestinal motility. Functional assays could represent useful tools to investigate the effects evoked by the entire probiotic cells and/or their fraction on different segments of the intestinal tissue.

The aim of this study was to evaluate the effect of the eight strains within the probiotic preparation VSL#3^[18,21-23] on intestinal motility using ileum and proximal colon segments isolated from guinea-pigs. Entire bacterial cells, cell fractions of VSL#3 strains, and, as controls, *Escherichia coli*, *Salmonella aboni* and *Bacillus licheniformis*, were tested in this *in vitro* model.

MATERIALS AND METHODS

Bacterial strains and culture conditions

The following bacterial strains were used in this study: *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum* and *Streptococcus thermophilus*.

Bifidobacterium and *Lactobacillus* strains were grown anaerobically (Anaerobic System, Model 2028, Forma Scientific, Marietta, OH, USA) in MRS medium (Difco, Detroit, MI, USA) supplemented with 0.05% L-cysteine hydrochloride monohydrate (Merck, Darmstadt, Germany), at 37°C. *S. thermophilus* was cultured anaerobically in M17 medium (Difco) at 37°C. *Salmonella aboni* NCTC 6017, *Escherichia coli* ATCC 11105 and *Bacillus licheniformis* BGSC 5A24 were used as controls and grown aerobically in LB medium (Difco) at 37°C.

Preparation of bacterial cell fractions

Ten milliliter of the bacterial mid log cultures, counted by plating technique on the previously mentioned media, was collected by centrifugation ($5000 \times g$ at 4°C for 5 min), washed, and resuspended in 1 mL of De Jalon buffer (155 mmol/L NaCl, 5.6 mmol/L KCl, 0.5 mmol/L CaCl₂, 6.0 mmol/L NaHCO₃, 2.8 mmol/L glucose)^[25], obtaining a final bacterial concentration of 1×10^9 - 1×10^{10} colony forming units (CFUs/mL). Subsequently the bacterial suspensions were sonicated (Bronson Sonifier W-250, Heineman, Schwäbisch, Germany) at power level 2 at 20% duty for 8 min and centrifuged at $8000 \times g$ for 30 min to separate cell debris from crude extract. Cell debris was resuspended in 1 mL of De Jalon buffer. The crude extract was ultracentrifuged at $250\,000 \times g$ at 4°C for 2 h: the supernatant represents the cytoplasmatic fraction while the pellet, resuspended in 1 mL of 50 mmol/L TRIS.HCl pH 7.6, represents the fraction enriched in membrane proteins.

In order to obtain the bacterial cell wall fraction, bacterial cultures were centrifuged at $5000 \times g$ at 4°C for 5 min, washed in 50 mmol/L Tris HCl pH 7.6, resuspended in 1 mL of protoplast buffer [50 mmol/L Tris HCl pH 7.6, 1 mol/L sucrose, 50 mL of completeTM protease inhibitor (Roche, Mannheim, Germany), 15 mg/mL lysozyme] and incubated at 37°C for 20 min. The supernatant was recovered by centrifuging at $5000 \times g$ at 4°C for 5 min.

Proteinaceous components were precipitated by addition of 9 volumes of acetone:HCl (10:0.1) to one

volume of the cytoplasmatic fraction and collected by centrifuging at $12000 \times g$ at 4°C for 5 min. Supernatant (non-proteinaceous fraction) and protein pellet were dried on ice under vacuum for removing acetone and resuspended in the initial volume of 50 mmol/L TRIS.HCl pH 7.6. The very low value of pH (about 1) of the non-proteinaceous fraction was adjusted to a value of 7.4 to overcome the unspecific colonic contractile response due to the acid pH. Similarly, the cytoplasmatic fraction was incubated with proteinase K (500 mg/mL) at 50°C for 1 h for the enzymatic digestion of the proteins. All the bacterial fractions of cell debris (i.e., cell wall fraction, membrane proteins, cytoplasmatic fractions, proteinaceous and non-proteinaceous cytoplasmatic components) were aliquoted and stored at -80°C before to be used in the *in vitro* stimulation of ileum and proximal colon.

SDS PAGE

Ten μ L of *B. infantis* cytoplasmatic fraction, non-proteinaceous fraction and cytoplasmatic fraction deprived of proteins by proteinase K digestion was analyzed by SDS-PAGE, as described by Laemmli^[26], using a 12% polyacrylamide running gel. The gels were stained with silver nitrate.

Bacterial DNA preparation

Isolation of genomic DNA from pure cultures of the probiotic bacteria was performed as previously described^[27]. In order to obtain the complete cell disruption, the method was slightly modified by prolonging the enzymatic lysis for 1 to 3 h and grinding with glass beads (150-212 μ m, Sigma, St. Louis, MO, USA). Concentration and purity of all DNA preparations were determined by measuring OD₂₆₀ absorbance and OD_{260/280} ratio, respectively. Only DNAs with an OD_{260/280} ratio > 1.8 were used.

Animals and preparation of Guinea-pig ileum and proximal colon

Guinea-pigs of either sex (200-400 g) obtained from Charles River (Calco, Como, Italy) were used. The animals were housed according to the ECC Council Directive regarding the protection of animals used for experimental and other scientific purposes. All procedures followed the guidelines of The Animal Care and Use Committee of The University of Bologna (Bologna, Italy). The animals were sacrificed by cervical dislocation, and the organ (ileum or proximal colon) required was set up rapidly under a suitable resting tension in 15-mL organ baths containing appropriate physiological salt solution (PSS) consistently warmed (see below) and buffered to pH 7.4 by saturation with 950 mL/L O₂ and 50 mL/L CO₂ gas.

Preparation of ileum

The terminal portion of ileum (3-4 cm near the ileo-caecal junction) was cleaned with Tyrode solution of the following composition: 118 mmol/L NaCl, 4.75 mmol/L KCl, 2.54 mmol/L CaCl₂, 1.20 mmol/L MgSO₄, 1.19 mmol/L KH₂PO₄·2H₂O, 25 mmol/L NaHCO₃, and 11 mmol/L glucose. The mesenteric tissue was removed. The ileum tissue was cut in two segments of 2-3 cm taken in the lon-

gitudinal direction along the intestinal wall. The segments were set up upright under 1-g tension in a jacketed tissue bath (15 mL, 37°C) containing Tyrode solution buffered to pH 7.4 by saturation with 950 mL/L O₂ and 50 mL/L CO₂ gas. Tissues were allowed to equilibrate for at least 30 min, during which time the bathing solution was changed every 10 min. After stabilization, the strips were challenged with 1 µmol/L carbachol (Sigma, Italy) to assess the responsive capacity of the tissue^[28].

Preparation of proximal colon

Starting approximately 1 cm distal from the caecocolonic junction, a segment of about 3 cm was excised, cleansed by rinsing it with De Jalon solution^[25], and the mesenteric tissue was removed. The proximal colon segment was cut in two segments of about 1 cm each taken in the longitudinal direction along the intestinal wall. The segments were set up upright under 1-g tension at 37°C in a jacketed tissue bath (15 mL, 37°C) containing De Jalon solution buffered to pH 7.4 by saturation with 950 mL/L O₂ and 50 mL/L CO₂ gas. Tissues were allowed to equilibrate for at least 30 min during which time the bathing solution was changed every 10 min. After stabilization, the strips were challenged with 5 µmol/L 5-hydroxytryptamine (5-HT) (Sigma, Italy) in the presence of 1 µmol/L atropine (Sigma, Italy) to assess the responsive capacity of the tissue.

Functional assays on ileum and proximal colon

After stabilization and assessment of the responsive capacity of the tissue in the organ bath, concentration-response curves were constructed by cumulative addition of the above described bacterial preparations (1 to 1500 µL). Each successive addition of bacterial preparations was performed after the response to the previous addition reached its maximum level and remained steady. Longitudinal muscle contractions or relaxations were recorded isometrically by securing one end of the tissue segments to a tissue holder and the other end to a force displacement transducer (FT. 03, Grass Instruments, Quincy, MA) using Power Lab software (ADInstruments Pty Ltd, Castle Hill, Australia). Each tracing was obtained by using separate intestinal strips.

Statistical analysis

Experiments were performed in duplicate with tissue from the same animal, and mean values were recorded. All data are presented as mean ± SE ($n = 3-5$ for ileum and $n = 5-7$ for proximal colon). Differences between means were calculated with Student *t*-test and *P*-values < 0.05 were considered statistically significant^[29-31].

RESULTS

Bacterial effect on motility response in guinea-pig ileum

The motility response of the guinea-pig ileum segment was investigated with increasing concentrations of the following components of the probiotic VSL#3 mixture: live bacteria, bacterial cell debris, and crude extracts. VSL#3 live bacteria and cell debris did not modify the guinea-pig ileum motility (data not shown), whereas crude

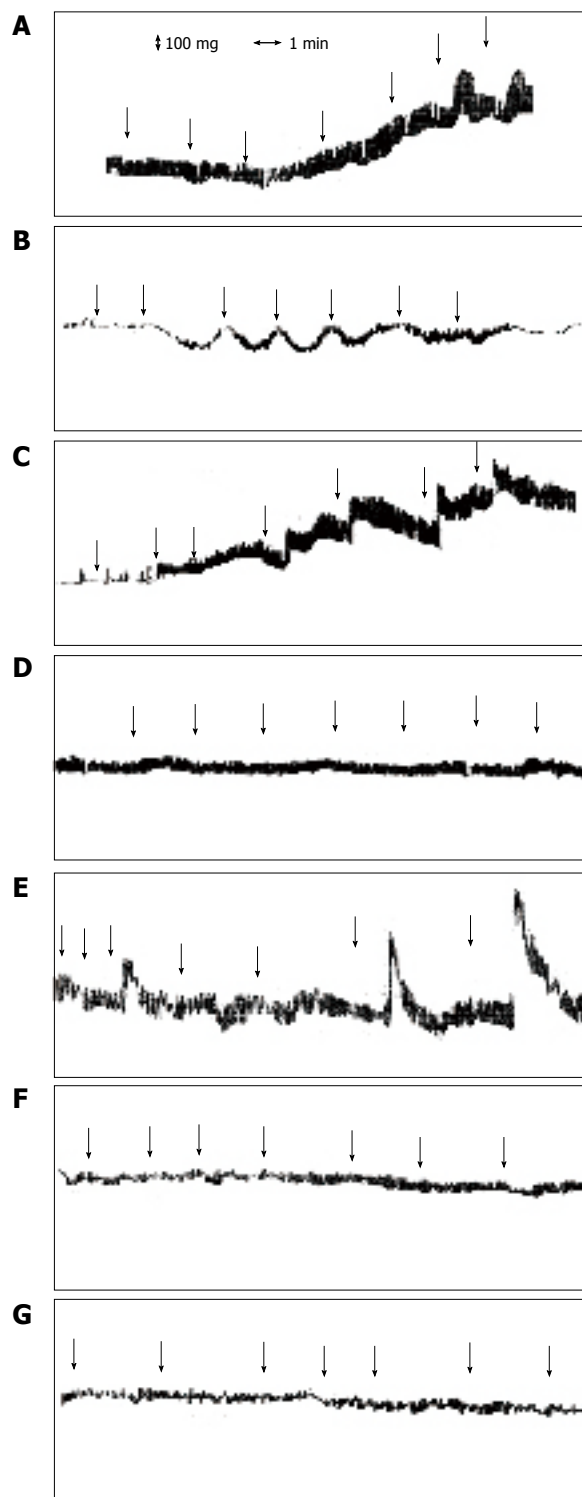


Figure 1 Recorder tracing of the cumulative dose of VSL#3 (A), *Bifidobacterium* (B), *Lactobacillus* (C), *Streptococcus thermophilus* (D), *Salmonella aboni* (E), *Escherichia coli* (F), and *Bacillus licheniformis* (G) crude extracts on the contractility of Guinea-pig isolated ileum. Arrows indicate the addition of 50, 100, 150, 200, 250, 300 and 350 µL of each crude extract tested.

extracts dose-dependently increased both spontaneous phasic and tonic contractions of the ileum (Figure 1A). The effects on motility induced by crude extracts persisted even after adjusting the pH of the solution from around 5.0, as found in the crude extract, to pH 7.4 and were reversible as the basal tone returned to initial values after

several washing steps (data not shown).

To identify the microbial genera within the VSL#3 cocktail responsible for the effects on motility in the guinea-pig ileum, crude extract mixtures of *Bifidobacterium* (*B. longum*, *B. infantis*, *B. breve*), *Lactobacillus* (*L. acidophilus*, *L. casei*, *L. plantarum*, *L. bulgaricus*), and *Streptococcus thermophilus* were individually tested. The following differences in the motility response by the crude extracts of the three probiotic genera were observed: (1) *Lactobacillus* strains induced a concentration-dependent contraction of the ileum, which was rapid in onset; (2) *Bifidobacterium* promoted a weak and transient stimulation of the ileum in a non concentration-dependent manner; (3) *Streptococcus* did not promote any significant response (Figures 1B-D).

The intestinal bacteria *Salmonella aboni* and *Escherichia coli* and the non-intestinal bacterium *Bacillus licheniformis* were used as controls to verify the specificity of the probiotic effects on gut motility (Figures 1E-G). Ileum motility was not affected by the exposure to *E. coli* and *B. licheniformis* crude extracts (Figures 1F, G), whereas *S. aboni* crude extracts were able to trigger the ileum contraction in a dose-dependent manner (Figure 1E). Furthermore, the amplitude of contraction induced by *S. aboni* was significantly higher than that promoted by the VSL#3 mixture and *Lactobacillus* crude extracts.

In order to verify the involvement of muscarinic receptors in the observed contraction response, the muscarinic antagonist atropine (1 $\mu\text{mol/L}$) was added to the organ bath. Atropine did not modify the motility response of ileal tissue exposed to all the bacterial crude extracts tested (data not shown).

Bacterial effect on motility response in guinea-pig proximal colon

The motility response of guinea-pig proximal colon segment was investigated using a similar protocol as with the guinea-pig ileum by adding identical concentrations of the bacterial cell preparations tested in the ileum stimulation study. Similar to the guinea-pig ileum, only the crude extracts of the probiotic VSL#3 mixture showed a significant effect on colon motility. However, the effect on the guinea-pig proximal colon was opposite to that observed in ileum (Figure 2A). In particular, the crude extract of the VSL#3 mixture elicited a rapid and sustained relaxation of the colon tissue, characterized by peaks in response to each addition, together with a progressive lowering of the basal tone. As previously carried out with the ileum, crude extract preparations at pH 5 and 7.4 were tested obtaining the same colon relaxation response (data not shown).

The VSL#3 extract mixtures of the genera *Bifidobacterium*, *Lactobacillus*, and *S. thermophilus* were assessed (Figures 2B-D). All the bacterial groups provoked a dose-dependent relaxation of the colon tissue, but no significant motility effect was observed by exposure to the control bacteria (Figures 2E-G). Clear colon relaxation effects were promoted by the crude extracts of *Bifidobacterium* strains and *S. thermophilus* and were quite similar to those induced by the crude extracts of the VSL#3 mixture (Figure 3). *Lactobacillus* strain's crude extract showed a lower relaxation

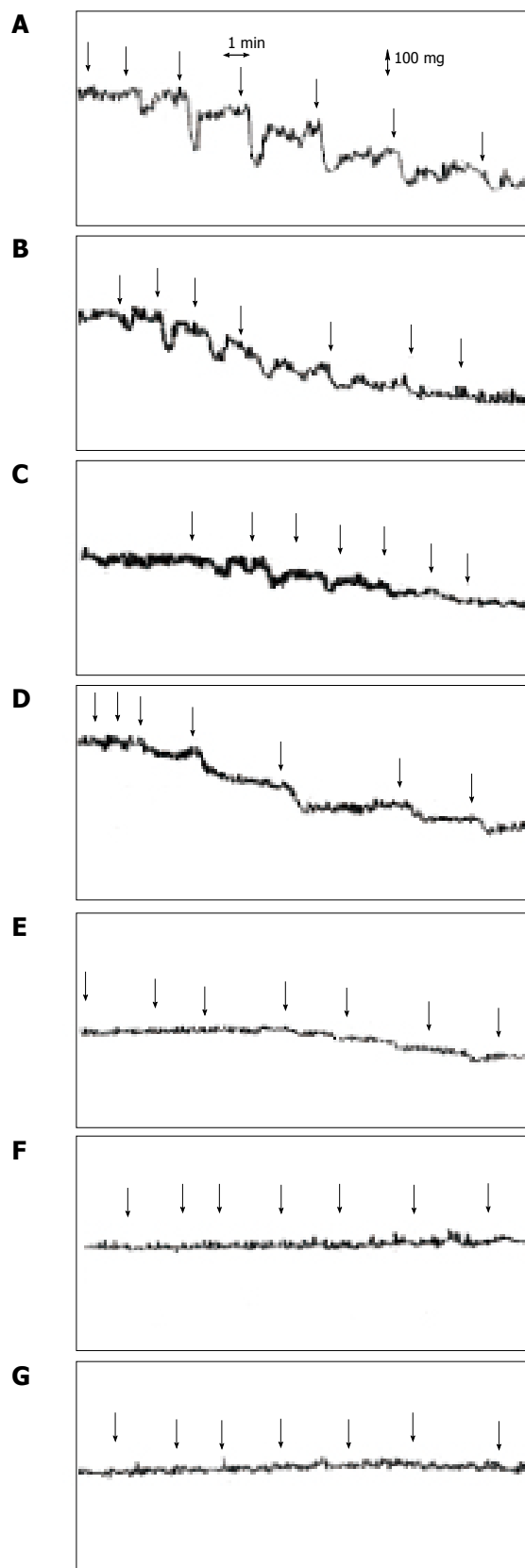
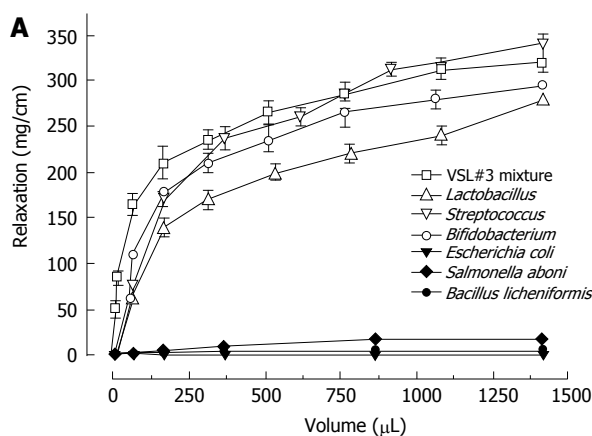


Figure 2 Recorder tracing of the cumulative dose of VSL#3 (A), *Bifidobacterium* (B), *Lactobacillus* (C), *Streptococcus thermophilus* (D), *Salmonella aboni* (E), *Escherichia coli* (F), and *Bacillus licheniformis* (G) crude extracts on the contractility of Guinea-pig isolated proximal colon. Arrows indicate the addition of 50, 100, 150, 200, 250, 300 and 350 μL of each crude extract tested.

response. Washings of the colon segment exposed to



Bacterial strains	μL	Relaxation %
VSL#3 mixture	1100	100
<i>Lactobacillus</i>	1400	86 ± 3.9
<i>Streptococcus</i>	1400	106 ± 7.5
<i>Bifidobacterium</i>	1050	92 ± 1.3
<i>Escherichia coli</i>	1400	0
<i>Salmonella aboni</i>	1400	0
<i>Bacillus licheniformis</i>	1400	6 ± 0.4

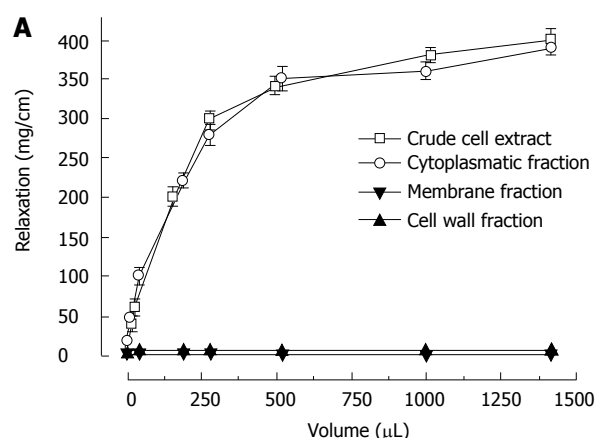
Figure 3 Relaxation induced by crude extract of VSL#3 mixture, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Escherichia coli*, *Salmonella aboni* and *Bacillus licheniformis* preparations on Guinea-pig proximal colon. Relaxation was expressed as change in mg-tension per cm of chart paper in resting tone (A). Relaxation was expressed in percentage, by considering VSL#3 mixture-induced maximal relaxation as 100% (B). The volume (μL) indicates the minimal value exerting maximal relaxation effects. Each crude extract was prepared from a bacterial suspension equivalent to a concentration of 3.5×10^5 CFUs/mL. Each point is the mean ± SE of 5 to 7 observations. Mean ± SE was given ($P < 0.05$).

successive additions of all crude extracts tested restored the basal tone of the tissue (data not shown). A further investigation was carried out with the crude extracts of VSL#3 *B. infantis* and *L. casei* strains, representative of *Bifidobacterium* and *Lactobacillus* genera. Colon relaxation patterns induced by these samples were identical to those obtained with the crude extracts of *Bifidobacterium* and *Lactobacillus* mixtures (data not shown).

Effects of *B. infantis* cell components on colon motility

To confirm that bacterial cytoplasmatic components are mainly responsible for colon relaxation, a more refined fractioning of the *B. infantis* cells was performed by separating cell wall fraction, membrane proteins, and cytoplasmatic fraction. A concentration-response curve was constructed for each of these bacterial portions (Figure 4). Indeed, the cytoplasmatic fraction showed a sustained relaxation of the colon segment equal to that demonstrated by the *B. infantis* crude extract. As expected, the cell wall fraction and the membrane proteins did not produce any significant effect.

The cytoplasmatic fraction of *B. infantis* was further refined in proteinaceous and non-proteinaceous components. The absence of protein in the non-proteinaceous fraction was verified by SDS PAGE analysis (Figure 5). As reported in Figure 6, the effect on colon relaxation was induced by the non-proteinaceous components, which showed a motility response similar to



<i>B. infantis</i>	μL	Relaxation %
Crude cell extract	1400	100
Cytoplasmatic fraction	1400	96 ± 3.5
Membrane fraction	1400	0
Cell wall fraction	1400	0

Figure 4 Relaxation induced by *B. infantis* crude extract, cytoplasmatic fraction, membrane fraction and cell wall fraction on Guinea-pig proximal colon. Relaxation was expressed as change in mg-tension per cm of chart paper in resting tone (A). Relaxation was expressed in percentage, by considering crude extract-induced maximal relaxation as 100% (B). The volume (μL) indicates the minimal value exerting maximal relaxation effects. Each crude extract was prepared from a bacterial suspension equivalent to a concentration of 3.5×10^5 CFUs/mL. Each point is the mean ± SE of 5 to 7 observations. Mean ± SE was given ($P < 0.05$).

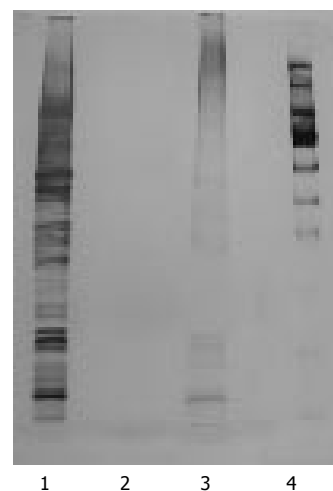


Figure 5 SDS PAGE electrophoresis of *B. infantis* cytoplasmatic fraction (lane 1), cytoplasmatic non-proteinaceous components (lane 2), cytoplasmatic fraction proteinase K digested (lane 3), molecular weight marker (lane 4).

that exerted by the cytoplasmatic fraction. The relaxation effect exerted by the non-proteinaceous components was confirmed by colon motility experiments carried out with a *B. infantis* cytoplasmatic fraction deprived of proteins by proteinase K digestion. This preparation, in which the enzymatic protein degradation was demonstrated by SDS PAGE analysis (Figure 5), evoked a significant colonic relaxation response similar to that obtained with the buffered non-proteinaceous preparation (Figure 6). To further characterize the *B. infantis* non-proteinaceous cytoplasmatic components, the possible involvement of bacterial genomic DNA fraction was tested. Addition to the organ bath of *B. infantis* DNA ranging from 0.5 μg to 10 μg did not modify the colon motility (data not shown).

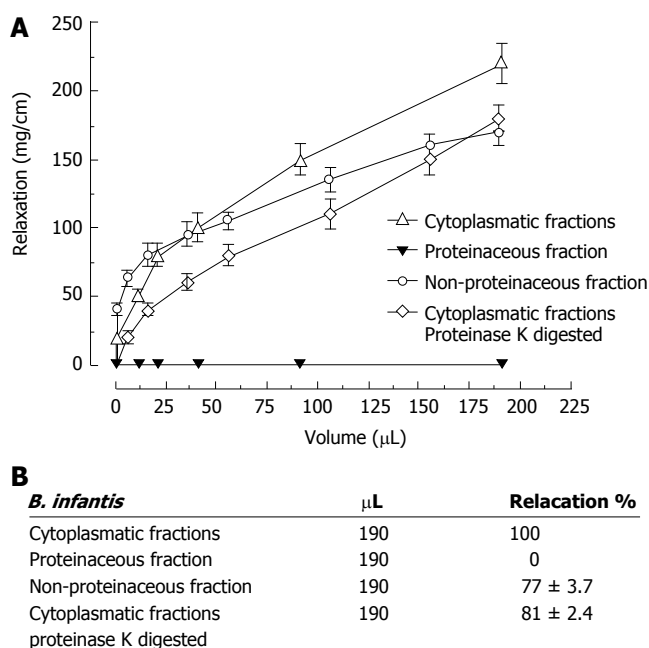


Figure 6 Relaxation induced by *B. infantis* cytoplasmic fraction, cytoplasmic proteinaceous components, cytoplasmic non-proteinaceous components and cytoplasmic fraction proteinase K digested on guinea-pig proximal colon. Relaxation was expressed as change in mg-tension per cm of chart paper in resting tone (A). Relaxation was expressed in percentage, by considering cytoplasmic fraction-induced maximal relaxation as 100% (B). The volume (μL) indicates the minimal value exerting maximal relaxation effects. Each crude extract was prepared from a bacterial suspension equivalent to a concentration of 3.5×10^9 CFUs/mL. Each point is the mean \pm SE of 5 to 7 observations.

DISCUSSION

The gut represents a complex and dynamic microbial ecosystem in which intestinal microflora has important and specific metabolic, trophic, and protective functions. Normal gut structure and function are the end-point of a complex set of interactions between the host and microorganisms colonizing the gut^[2]. Several studies have shown that probiotics restore mucosal integrity and regulate the immune response. However, the effects of probiotics on the enteric nervous system and the intestinal musculature have not been systematically studied. This study was designed to investigate the motility response evoked by the probiotic preparation VSL#3 on the ileum and proximal colon isolated from guinea-pigs.

The choice of VSL#3, a formulation with a high viable concentration of *Bifidobacterium*, *Lactobacillus* and *Streptococcus thermophilus*, was based on the reported efficacy in bowel diseases associated with changes in propulsive motility of the gut, including diarrhea-predominant irritable bowel syndrome^[18]. Furthermore, in clinical trials these exogenous probiotic bacteria have been shown to transiently colonize the human gut^[19, 21, 22].

The data from this study show that the motility of isolated ileum is not influenced by the addition of either entire cells or cell debris of the VSL#3 mixture, whereas its crude extracts induce a dose-dependent contraction. It is noteworthy that VSL#3 crude extracts, which have a pH of about 5, and crude extracts in which the pH was increased up to physiologic value of 7.4 showed

similar contractile responses. These results demonstrate that the ileum motility response is not related to the pH of the bacterial fraction and suggest that some bacterial metabolites could be involved in the contractions. Furthermore, as the presence of atropine (1 μmol/L) in the organ bath throughout the experiments did not determine a decrease of contraction induced by the addition of the VSL#3 crude extracts, the mechanism of action of the bacterial fractions does not involve muscarinic receptors.

Crude extracts of the different bacterial groups within the VSL#3 preparation provoke different motility response patterns. *Bifidobacterium* and *Streptococcus* strains did not change the basal tone of the ileum tissue, while *Lactobacillus* strains induced relevant ileum contractions. However, this contractile response is observed with volumes of cell extracts corresponding to *lactobacilli* concentration values of at least 10^7 CFU/mL, a value that is generally not detectable at ileum level of healthy humans^[27]. This result suggests that the contractile effect evoked in ileum by VSL#3 cell extracts will be of minor importance *in vivo* at ileum level, as the rapid transit of ileum content and the low bacterial titer do not allow it to reach sufficient bacterial lysis or metabolic secretion. Furthermore, it is noteworthy that the contractile response induced by *Lactobacillus* is lower than that promoted by crude extracts of intestinal *S. aboni* added at the same concentration.

Like the guinea-pig ileum, the motility of guinea-pig proximal colon segment is modified by crude extracts of the VSL#3 mixture, while entire bacterial cells and cell debris do not exert any effect. The crude extract of all the probiotic strains (*Bifidobacterium*, *Lactobacillus* and *S. thermophilus*) included in VSL#3 induced dose-dependent relaxation of the proximal colon tissue, whereas the crude extracts of the control strains did not influence colon motility. These results suggest that the ability to induce a colon relaxation response is specific for these probiotic bacteria. Furthermore, the proximal colon relaxation response induced by probiotic bacteria cell extracts is reversible as successive washings abolished the colon relaxation. The restoration of the basal tone of the proximal colon tissue after removing the stimuli by several washings suggests that colonic tissue is not damaged. Volumes of cell extracts from *Bifidobacterium*, *Lactobacillus* and *Streptococcus* inducing the relaxation response correspond to bacterial titer values that are in the range of the physiological concentrations measured in colon^[19].

Since *B. infantis* has been shown to colonize the human gut more efficiently than the other VSL#3 bacterial strains^[22], we used this strain to further investigate the components of probiotic bacteria involved in the guinea-pig colon relaxation. Interestingly, only the cytoplasmic fraction of the cell showed a pronounced colon relaxation activity. The bacterial cytoplasmic fraction may well have physiological implications, because its specific components reach the intestinal lumen as metabolites actively secreted by the viable bacteria colonizing the intestinal epithelium. In addition, the entire bacterial cytoplasm is released in the gut lumen following cell lysis. Further, subdivision of the bacterial cytoplasm allowed us to demonstrate that the

non-proteinaceous cytoplasmatic portion was responsible for the colon relaxation, whereas protein factors had no effect on proximal colon relaxation. This result has been confirmed by challenging the colon tissue with bacterial cytoplasmatic fractions digested with proteinase K.

Since the cytoplasmatic fraction deprived of the protein component contains significant amounts of genomic DNA, which in prokaryotic microorganisms is not confined to a nucleus, the proximal colon segment was tested with the bacterial genomic DNA. The DNA preparation extracted from *B. infantis* did not cause any changes in colonic motility, indicating that other unidentified non-proteinaceous factors may be involved.

In conclusion, this is the first study performed in isolated tissues that deals with the effects of probiotic bacteria on intestinal motility. Our results suggest that *Bifidobacterium*, *Lactobacillus* and *Streptococcus* strains in VSL#3 mediate proximal relaxation activity. This could be one of the possible mechanisms of action by which probiotic bacteria exert their effects in ameliorating diarrhea by reducing stool frequency and restoring a disturbed microbial balance. Based on these data, further studies to elucidate the implication of a large array of receptor families and subtypes known to affect gut function and to characterize the non-proteinaceous bacterial molecules involved in intestinal motility are warranted.

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